

A microscopic image showing a large number of green, rod-shaped Bacillus spores. The spores are scattered across the field of view, with a higher concentration in the center-right area. The background is a light blue-grey color.

Surface Sampling of *Bacillus* spores

NIOSH DREAM Workshop

Surface Sampling/Biomonitoring Breakout Session

November 13, 2008

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Acknowledgments

- **Kenneth D. Cole, PhD, Sandra Da Silva, PhD**

Bioassay Methods Group
Biochemical Science Division



- **Andy Persily, PhD**

Indoor Air Quality and Ventilation Group
Building Environment Division



- **Steven Choquette, PhD**

BioAssay Methods Group Leader
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- **Laurie E. Locascio, PhD**

Chief, Biochemical Science Division

- **Bert Coursey, PhD**

Department of Homeland Security



Efforts in Standards for Biological Countermeasures

Standards

Detection

Reference Materials

Testing/
Sampling

Guidance

Training

Training Materials

ASTM E2458 /AOAC

Standard Sampling Methods
Decontamination

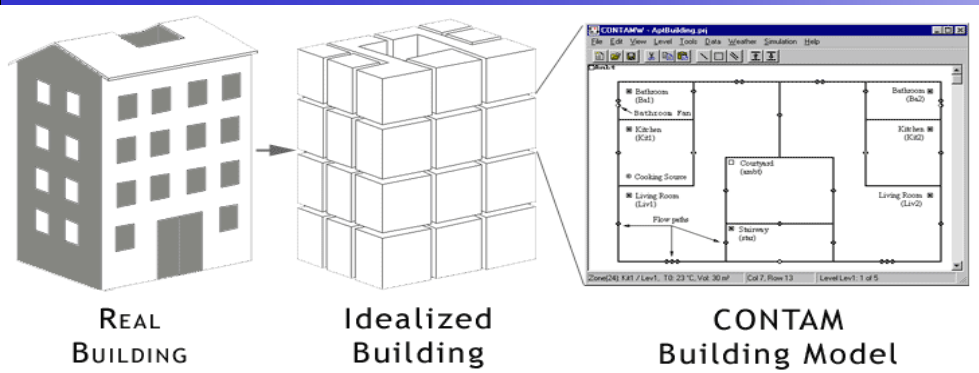
Surface Sampling Validation
CDC and EPA

Sampling Procedures for First Responders



**ASTM E2458/AOAC Standard effort
Led by Laurie Locascio**

- Produce Guidance for the Sampling of Nonvisible Surface Contamination
- Develop a strategy for determining the extent of contamination and decontamination effectiveness



**CONTAM/VSP Modeling Software:
Andy Persily at BFRL,
Greg Piepel and Brent Pulsipher at PNNL**

Sampling Visible Powders

The Goal: To develop the first US National Standard for collecting suspected biological agents.

The Motivation: Motivated by 2005 GAO report calling for validated methods for collecting suspected biological agents.

The Purpose: Reduce exposure risk, minimize on site sample consumption, reduce variability (sample handling and analysis).

The Scope: Collection of visible powders, credible biological threat for all biological agents from solid, nonporous surfaces, dispersed in a limited area

ASTM E2458 /AOAC Standard

Includes

- Response Coordination
- Field Screening
- Bulk Powder Sample Collection
- Transportation and Packaging

Bulk Powder Collection



Sterile plastic laminated card used with swab in “broom and dustpan” collection approach

Produces at least 2 samples that are transported to LRN (dry swab in tube, bulk powder in tube)

VSP Work Group

Validated Sampling Plan Work Group is a **DHS led multi-agency work group** set up to address the GAO request.

In a 2005 report titled **Anthrax Detection: Agencies Need to Validate Sampling Activities in Order to Increase Confidence in Negative Results (GAO-05-251)**, the Government Accountability Office (GAO) recommended that “to improve the overall process for detecting Anthrax and to increase confidence in negative test results...[DHS] ensure that the overall process of sampling activities, including methods, is validated so that performance characteristics, including limitations, are clearly understood and results can be interpreted.”

The whole sampling process is currently under validation by the CDC, EPA, FBI, NIST, DOD and other agencies.

Current Sampling Methods: Wipe and Swab Procedures

- Deposition method: aerosolized^{a,b} (95% Ethanol)
- Wetting agents: Water^{a,b} (PBS)
- Controlled Substrata: stainless steel^a, painted wallboard^a
- Wipes: rayon/polyester blend^a, rayon^b
- Extraction from Wipe: sonication^a, vortexing^b
- Report +/- Growth^b, reference coupons^{a,b}

Wipe Efficiency Ranges from 7 to 87%

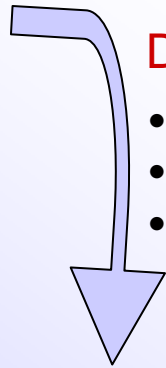
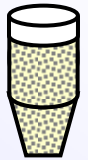
^aBrown, G. S., R. G. Betty, et al. (2007). "Evaluation of a Wipe Surface Sample Method for Collection of Bacillus Spores from Nonporous Surfaces." *Appl. Environ. Microbiol.* 73(3): 706-710.

^bSanderson, W. T., M. J. Hein, et al. (2002). "Surface Sampling Methods for Bacillus anthracis Spore Contamination." *Emerging Infectious Diseases* 8(10): 1145-1151.

Challenges in Surface Sampling Methods

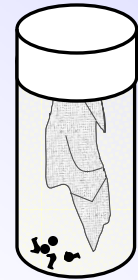
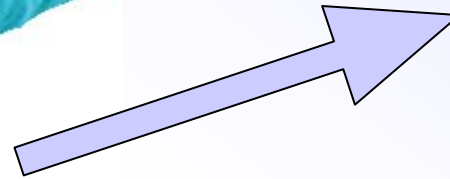
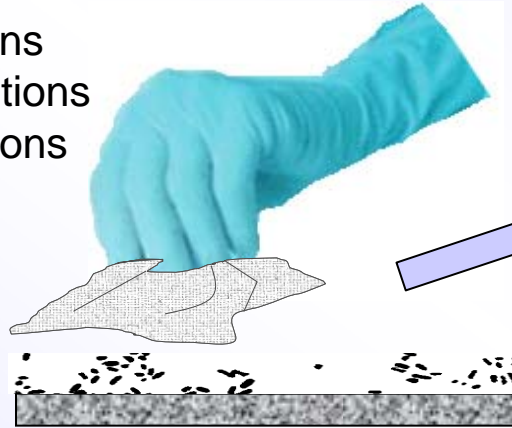
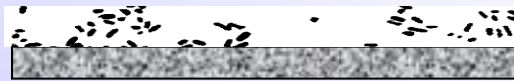
Spore Materials

- Reaerosolization
- Suspension stability
- Viability
- Quantity



Optimization of Deposition Method

- Solution conditions
- Deposition conditions
- Material interactions



Optimization of Sampling Method

- Environmental Conditions
- Sampling pressure and velocity
- Mass balance on material for loss evaluation
- Wipe and substratum material interactions
- Post-decon impacts on wipe efficiency

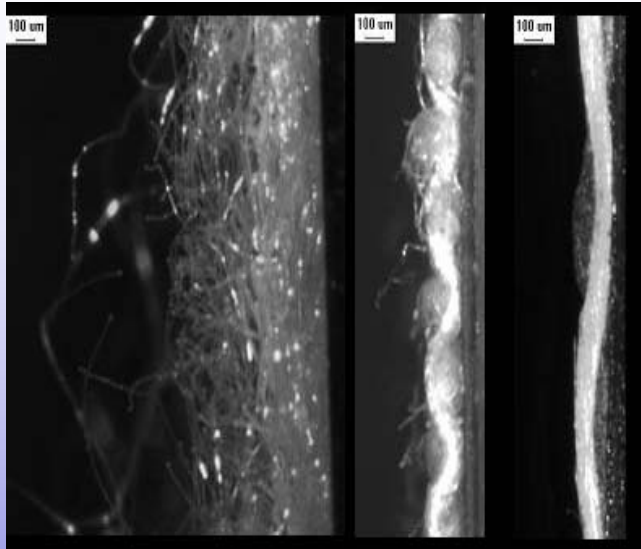
Integration with Detection Technology

- Optimization of removal from wipe
- Interference with detection technologies
- Post-decon impacts on wipe extraction

Why Develop a Dynamic Wipe Efficiency Method?

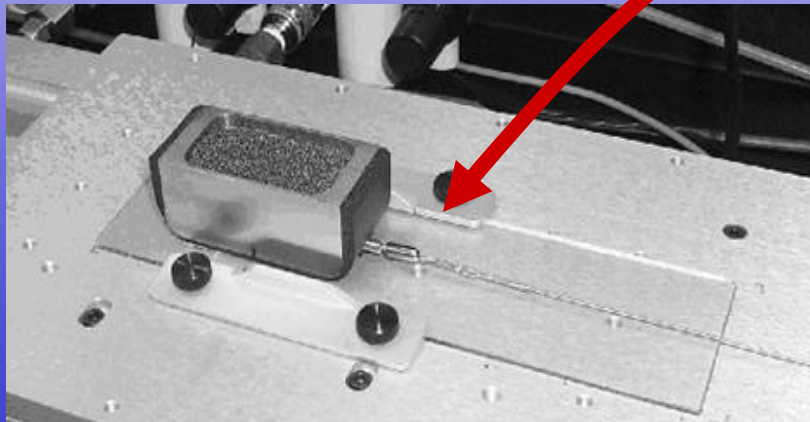
- Standard method to evaluate wipe performance in a controlled environment
- Address some of the challenges in surface sampling standards development
- Means for validation of:
 - New materials
 - Integration with new detection technology
 - Address losses and identify gaps in current capabilities
 - Different contaminants and contaminants housed in matrix materials

Standard Surface Sampling Method to Determine Dynamic Wipe Efficiency

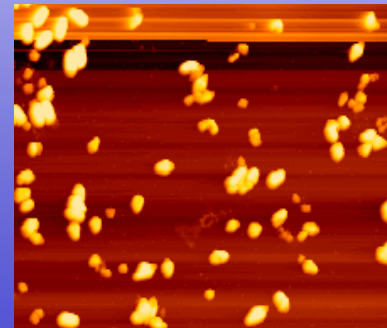


Nonwoven (Swiffer) Muslin PTFE

Controlled sampling procedure to evaluate wipe performance in controlled environment



Crockmeter Testing Device



Spores Deposited on Control Surfaces

Modeled on the successful efforts of the **NIST Trace Explosives Group**
Metrology of Explosive Particles and Vapors
Sampling, Detection, and Standards

Standard Method for Dynamic Wipe Efficiency

1. Material Characterization

Well characterized aqueous spore suspension



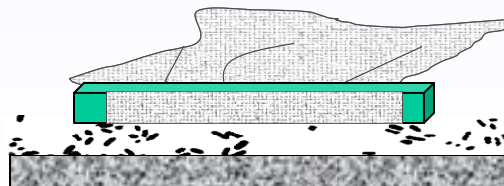
2. Deposition

Optically and AFM quantify/characterize deposited spores



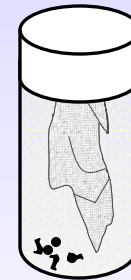
3. Wipe Sample

Wipe surface using crockmeter in environmental chamber



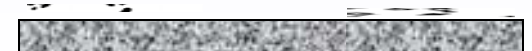
4. Extraction

Enhance extraction efficiency with solution chemistry manipulation



5. Performance

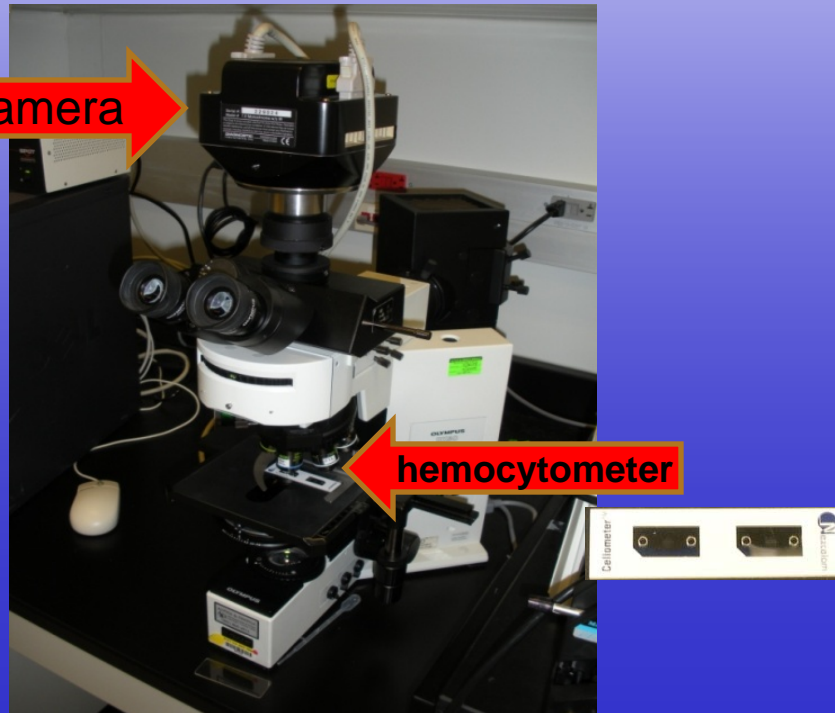
Optically quantify/characterize residual spores



Material Characterization: Spore Quantification

Direct Counts of Preparation Purity

Phase contrast microscopy used to manually count spores on a hemocytometer

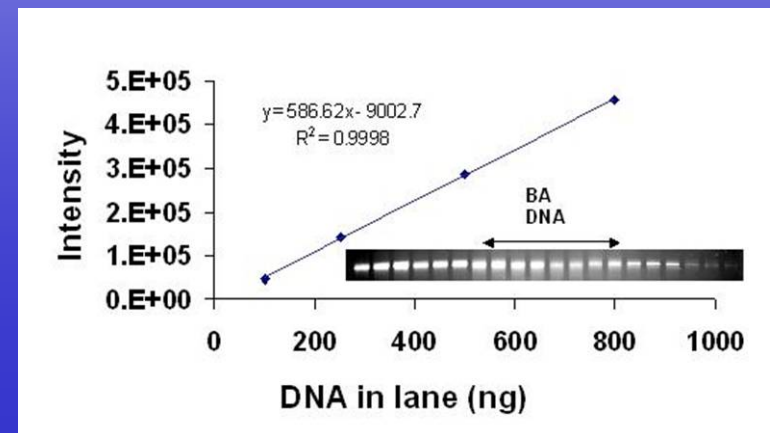


Viability Determination

LB agar plates were spread using serial dilutions and colony forming units (cfu)

DNA Quantity

PCR and gel electrophoresis are used to DNA concentration

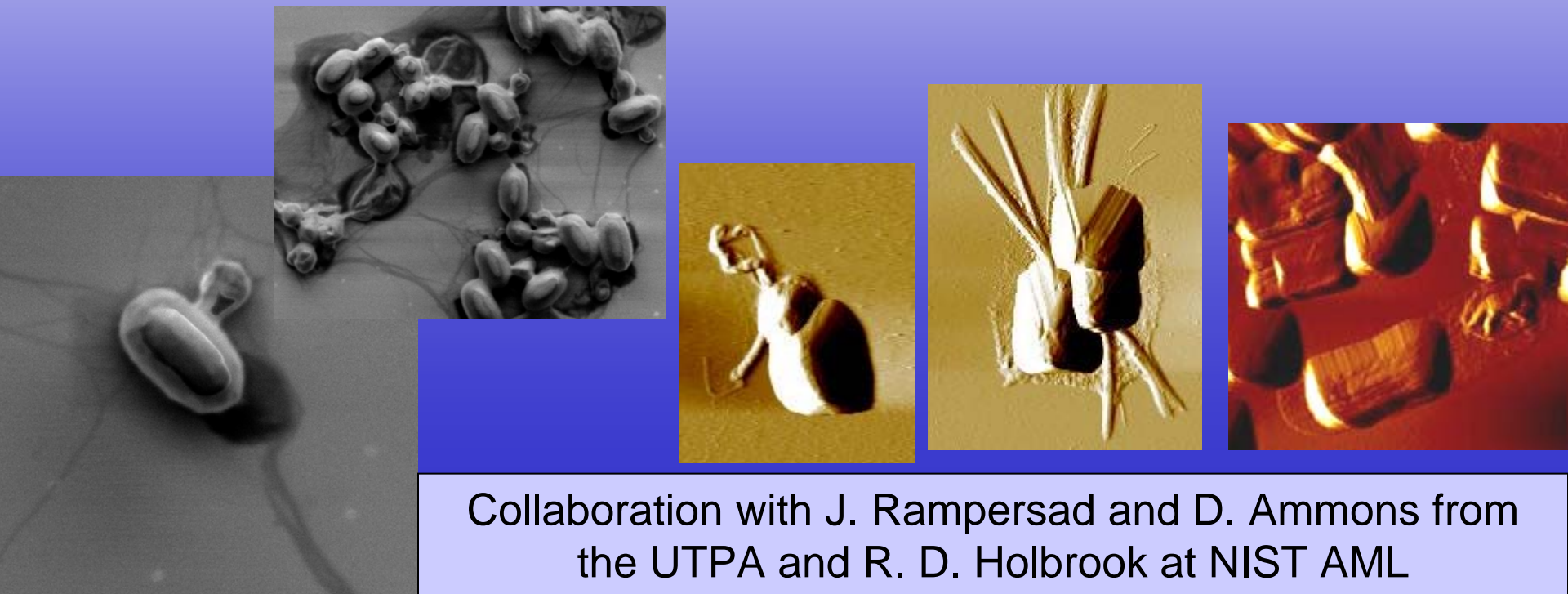


Material Characterization: Spore Aggregation

Measurements to aide in reference material production and understanding adhesion of spores to surfaces

- Surface Charge
- Hydrophobicity
- Particle size and morphology

} Colloid stability theory to understand aggregation and surface association

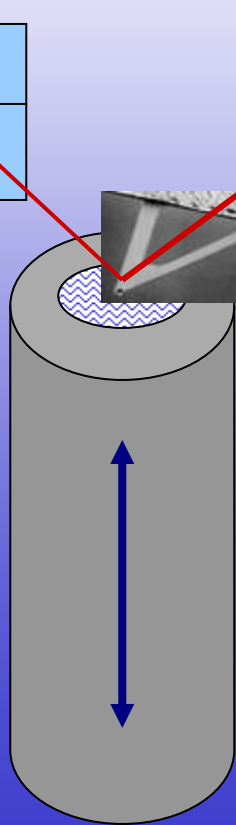
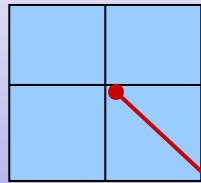


Collaboration with J. Rampersad and D. Ammons from the UTPA and R. D. Holbrook at NIST AML

Deposition Technique: Evaluate by Contact Mode AFM

Photodiode Detector

Laser Source

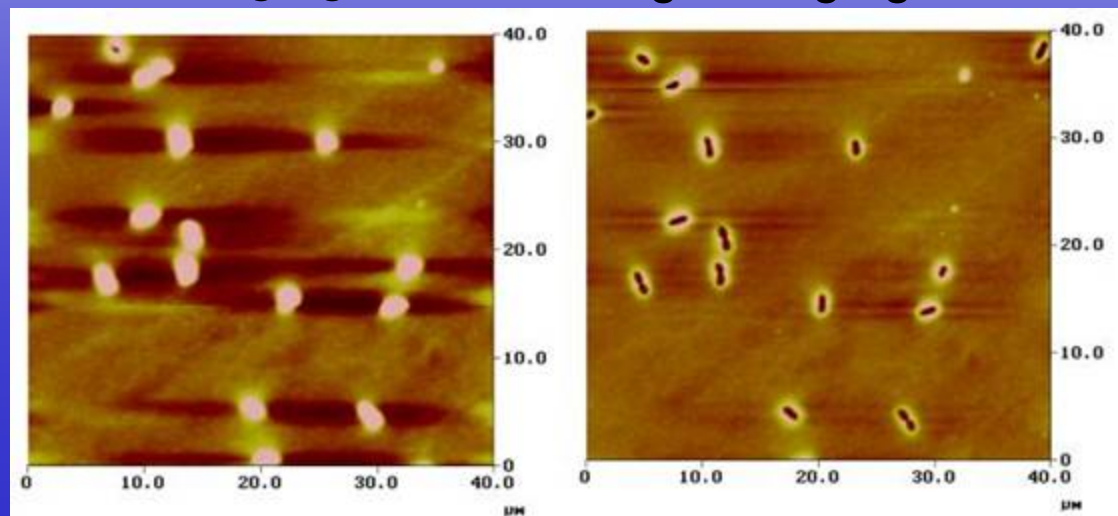


Evaluate Sampling Force Required Given:

- Humidity (RH of 40 - 90%)
- Surfactant (H₂O, volatile Buffer, \pm Tween 80)
- Substratum (Glass, SS, Teflon)

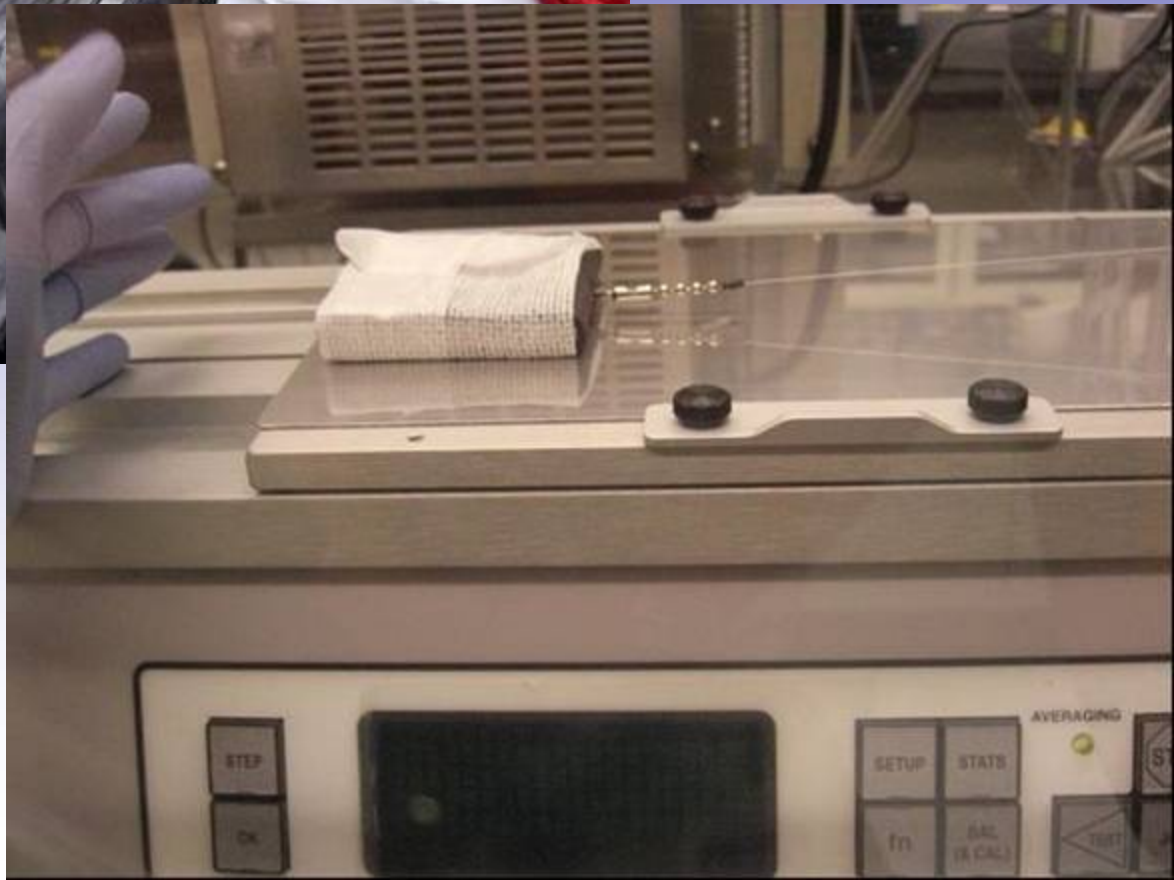
Low Imaging Force

High Imaging Force



Surface Sampling Test Parameters

- Deposition method
- Number of sampling passes
- Substrata: stainless steel, copper, aluminum, glass, Teflon
- Wipes:
 - Woven: cotton, polyester
 - Nonwoven: Versalon (rayon/polyester blend)
- Environmental factors
 - Relative humidity (40 to 90%)
 - Wetting Agent (DI water, PBS, \pm Tween 80)
 - Temperature
- Spores embedded in matrix materials
 - Urban particulate dust
 - Arizona dust
 - Skin cells



Overview of Efforts at NIST

Measurement Services for Characterized Reference Materials

Detection Technology Evaluation and Surface Sampling Science

- DNA Signatures
- Viability Determination
- Optical Characterization
- Surface Chemistry
- Suspension Stability

Guidance document for 1st Responder Technology Selection

Integration of Sampling with Detection Technologies

- DNA Signatures
- Antigenic Signatures
- Viability Determination
- Optical Characterization



Surface Sampling Science

ASTM method for sample collection

ASTM method for wipe efficiency

Guidance document for 1st Responder Surface Sampling

Biological Detectors for First Responders

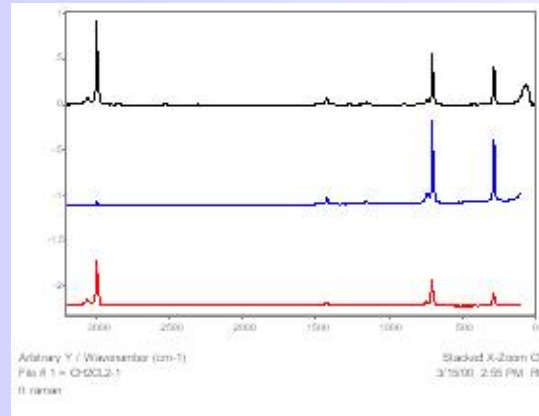
	Representative Technologies	Basis of the Technology
Screening Devices	Hand Held Assays, Nonspecific particle detection	Antigens, Nucleic acid signatures (non-PCR), Proteins Light Scattering/Fluorescence
Field Deployable Detectors	PCR Kit or Integrated PCR Immunological kit	Antigens, Nucleic acid signatures
Autonomous Monitors	Automated PCR, Aerosol detector	Nucleic acid signatures, Spectral properties (fluorescence, Raman)
Laboratory Analysis and Forensics	Standard methodologies (LRN), MIDI, Spectroscopy (FTIR, Raman)	DNA specific (PCR), Fatty Acid, Immunological, Culture Light Spectroscopy

Standards Development for Biothreat Detection by First Responder Community

- Spore reference materials for hand held detection assays
- Spore DNA reference materials for PCR based assays
- Raman spectroscopy reference materials and R&D related to reference material development



Raman SRM Development



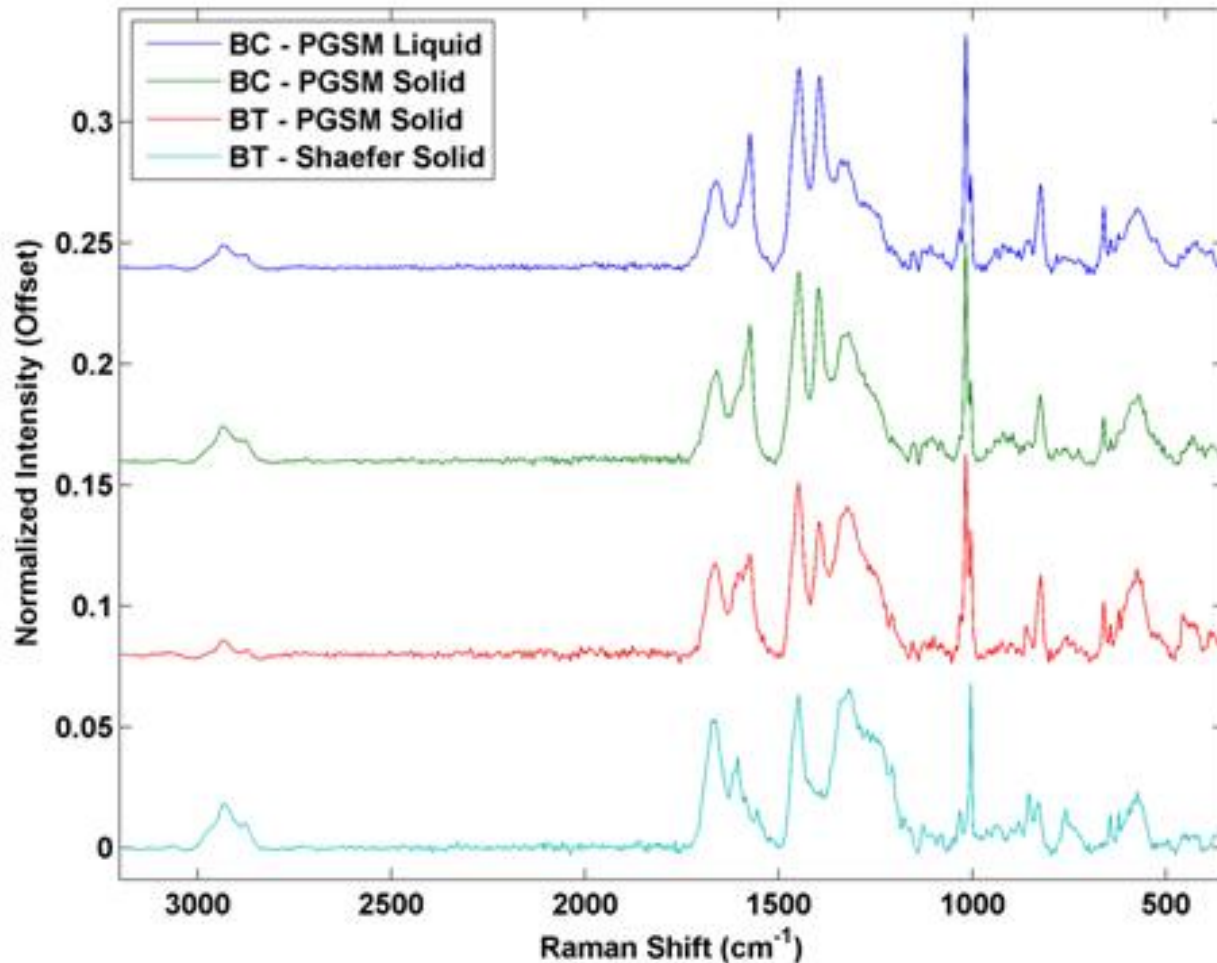
Raman Spectroscopy provides sample identification in real-time without sample preparation and through common containers.



Development of SRM's for calibration of both Raman Shift (x axis), Intensity (y axis) and Resolution.



Raman Spectroscopy- Growth Media Impact and Strain Variation



~60 mW, 785 nm excitation through a 50X objective on our Renishaw system

Spore/DNA RM Development

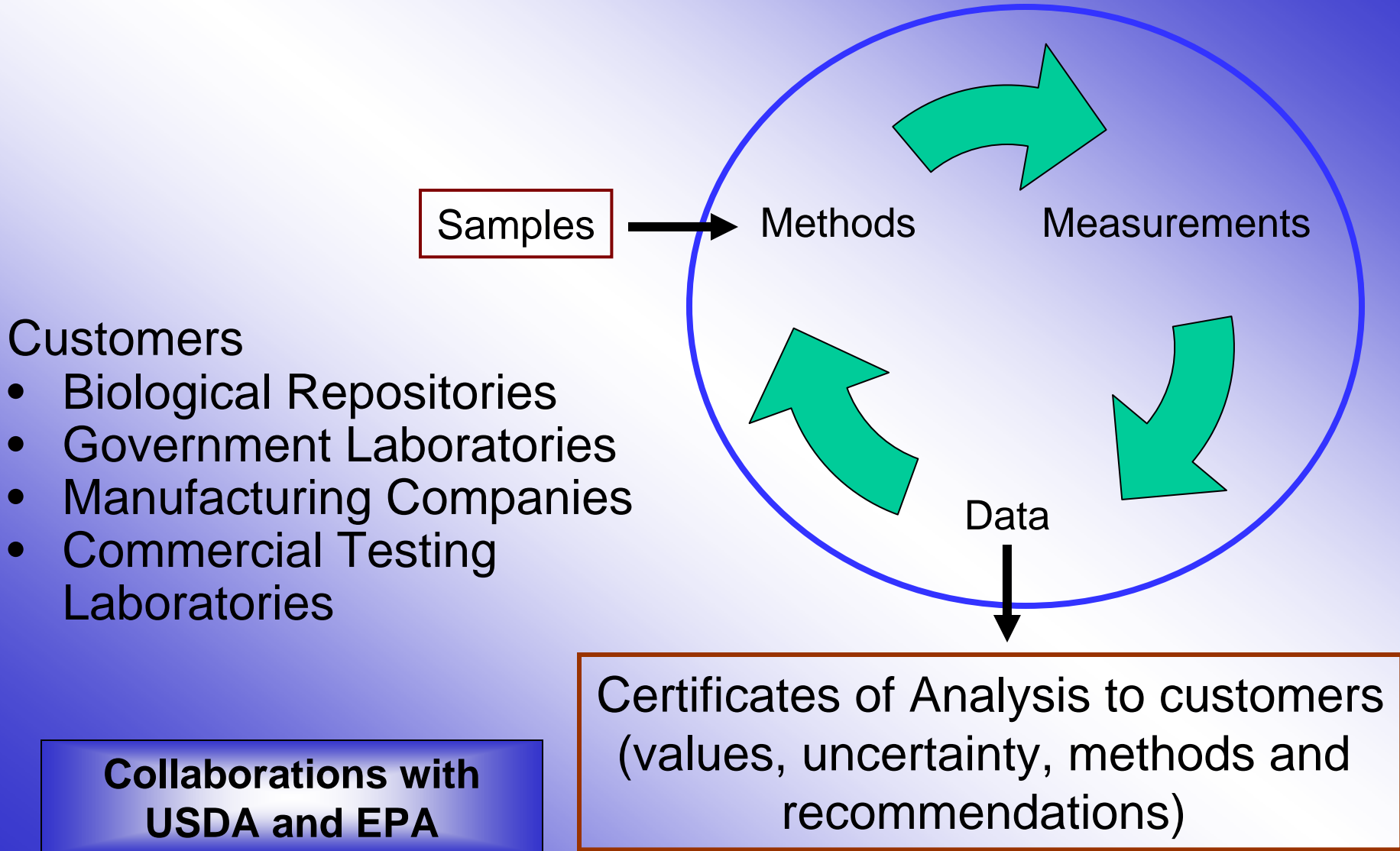
Development of Reference Materials for calibration of detector sensitivity and specificity.

Measurements to support RM development:

- Storage condition impacts on long term stability of spore suspension viability
- Quantification of spore preps
- Spore suspension stability and aggregation factors



Measurement Services



Future Efforts at NIST

- Improved methods for characterization of reference and training materials
- Measurement characterization of threats and rational selection of simulants and prediction of pathogen fate and transport
- Improved methods for detection or measurement of biological threats and threats in matrix materials including soils, particulate matrices
- Decontamination- data on disinfectants, improved disinfectants and methods for decon
- Sampling and integration of sampling with detection, decon and training on sampling methods

ACS Environmental Division Call for Papers

We would like to draw your attention to an upcoming symposium for the 238th American Chemical Society National Meeting in Washington DC from August, 16-20, 2009. This symposium within the Division of Environmental Chemistry is titled “**Detection and Sampling for Biodefense**”. The official Call for Papers can be found at <http://www.environfacs.org>.

The purpose of this session is to foster innovative interdisciplinary approaches to studying the integration of biological detection and sampling technologies by attracting researchers with a variety of scientific backgrounds who are studying this and related topics. This session will present recent advances in (i) innovative quantitative or mechanistic detection technologies, (ii) measurement issues in the integration of detection technology with surface and aerosol sampling methodologies (ii) bioanalysis in complex and environmental matrices and (iii) surface chemistry contributions to detection and sampling efficiency. We are particularly interested in work that evaluates the challenges in detection of specific microbial pathogens and biotoxin analytes relative to the biodefense community. Presenters are required to submit a short abstract to the ACS by March 16, 2009, using the ACS on-line system (OASYS) at <http://oasys.acs.org/>. **OASyS opens on January 19, 2009 and closes March 16, 2009.** There is no requirement for an extended abstract.

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