The Third Annual DHS Network Summit

Panel 23: Advancing Exposure Science

March 19, 2009: 1:45 PM – 3:15 PM

Project I. Exposure: Detection, Fate and Transport of Agents: Chuck Gerba

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Project 1

Exposure: Detection, Fate and Transport of Agents

Project 2

Infectious Disease Models for Assessing Microbial Risk and Developing Control Strategies

Project 3

Dose Response Assessment

Project 4

The Assessment-analysis Interface

Project 5

Knowledge Management, Transfer, and Learning

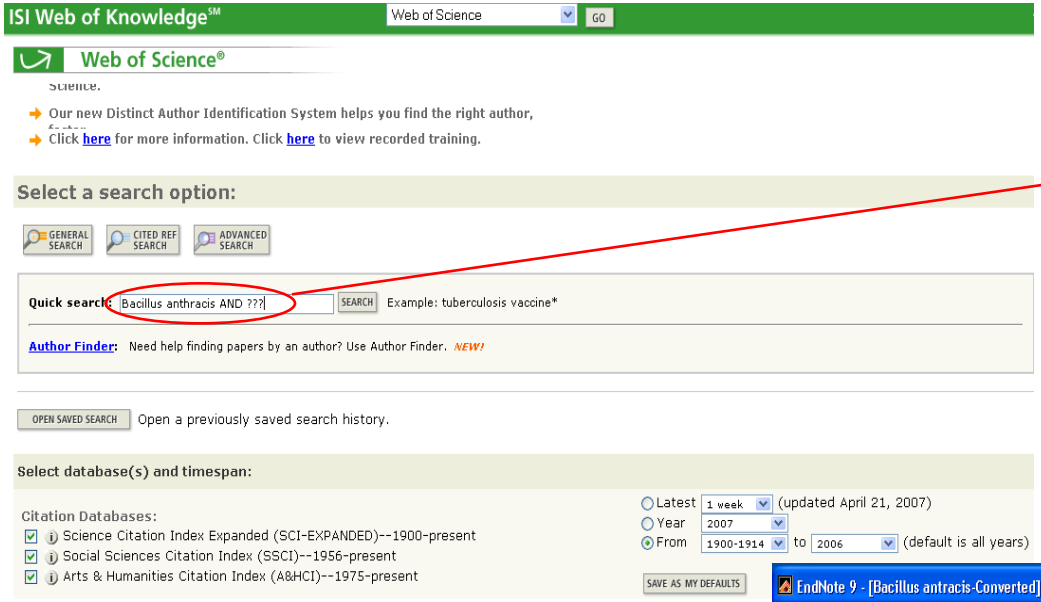
Project 1 Objectives

- Improve our ability to quantify exposure to biological agents of concern (BAC)
- Development of BAC surrogates
- Validation of detection methods

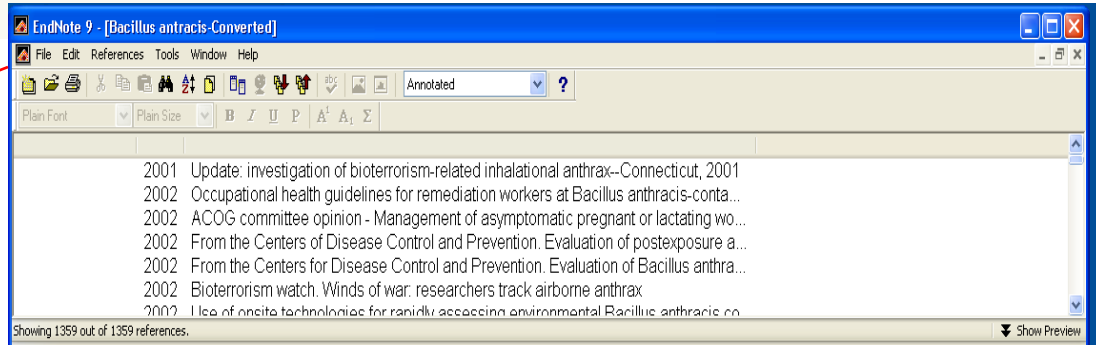
Outline

- **Project Objectives**
- **Literature Review for detection limits of *Bacillus anthracis***
  - Instrument Detection Limit
  - Environmental Detection Limit
  - Quantifying Limits of Risk Estimates
- **Fomite Recovery Study**
  - Sampling Techniques
  - Surface Area and Drying Time
  - Impact of Wetting Agent at Varying Relative Humidity
- **Conclusions**

Detection limit of various methods for *Bacillus anthracis*



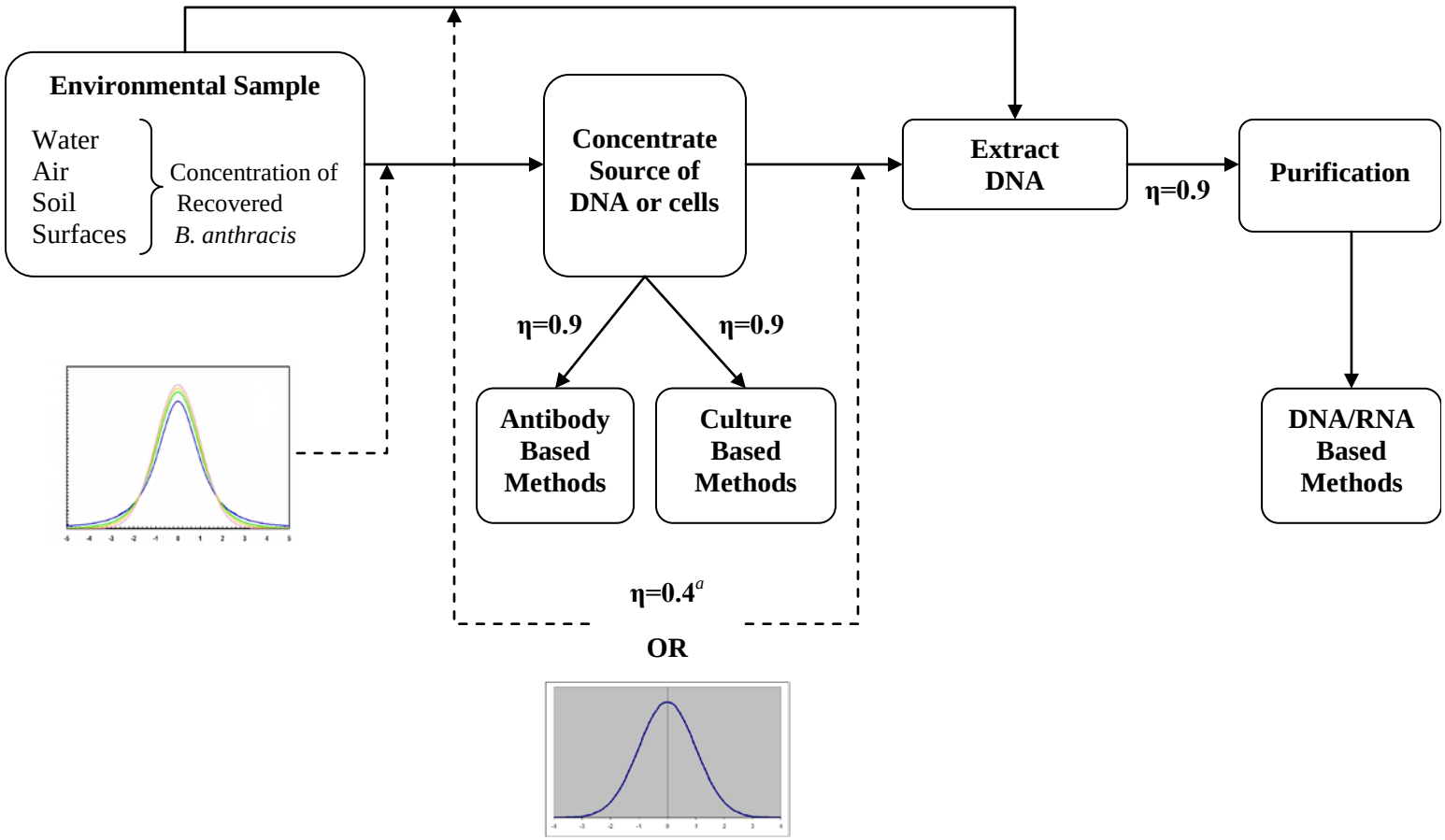
KEYWORDS: method, sensitivity, limit of detection, detection limit, limit, water, air , soil, fomite, surface, specificity, PCR sensor, environmental, rapid, assay, diagnostic, immunoassay, antibody, real time, real-time PCR, microfluidic, polymerase, quantitative, bioaerosol, aerosol, microarrays, biosensor, electrochemiluminiscence, Raman spectrometry, and mass spectrometry.



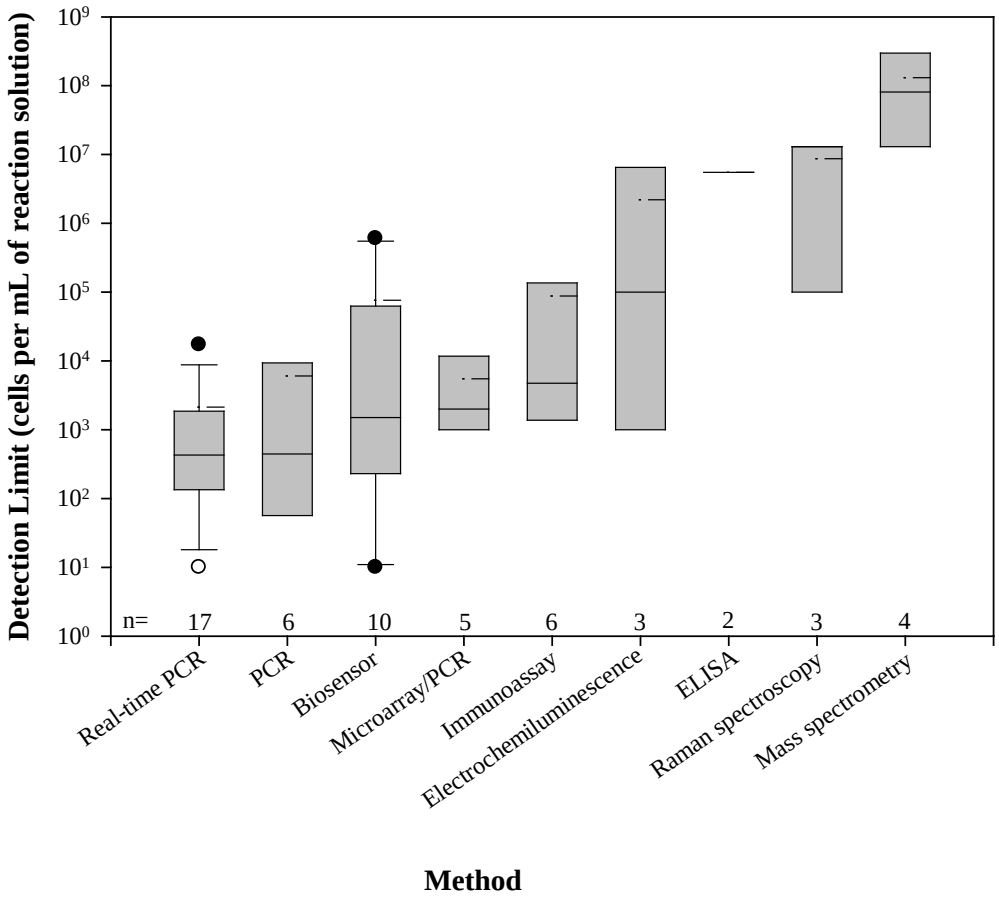
Detection Method	Sample Concentration	Pretreatment/Extraction	LOD

Efficiency of Sample Processing

Instrument Detection Limit vs. Environmental Detection Limit



Instrument Detection Limit of *Bacillus anthracis*



Most rapid and sensitive methods

- Real-time PCR: 430 cells/mL
- PCR: 440 cells/mL

Least sensitive methods

- Raman spectroscopy: 1.0x 10^7 cells/mL
- Mass spectrometry: 8.0x10^7 cells/mL

Statistical distributions could occur in the real-time PCR and biosensor methods

- No statistical distributions for n<10
- Misinterpretation of method detection capabilities
  - i.e. electrochemiluminescence when n is low and there is a wide distribution

Environmental Detection Limit of *Bacillus anthracis*

Soil

Number of Spores Spiked

Amount of Soil
(100g-1mg)

Soil Type
- Anthony fine sandy loam
- Cultivated
- Garden soil
- Garden soil w/ 3% peat
- Lawn
- Litter
- Meadow
- Sand
- Swamp

Soil Location
- Agriculture fields
- Contaminated sites w/ organic compounds and tanning agents
- National Institute of Health-Korea
- Non-suspicious sites
- Military fields

Pretreatment/Extraction Method
- Aqueous polymer 2-phase system
- Easy DNA Kit
- FastDNA SPIN Kit
- Cultivation in TSB
- 2x cultivation in TSB
- Hot Detergent/beadmill Homogenization
- Heat Treatment w/ 1.22g/mL sucrose/ 0.5% TritonX-100
- Heat Treatment w/ sterilized water and 10% TritonX-100/PBS
- 3 Freeze thaw cycles/ Glass beads and Glassmilk
- IM separation twice and resuspend in PBS

Detection Method
- Biosensor Assay
- IM-Electrochemiluminiscence
- Immunofluorescence Assay
- PCR
- PCR-ELISA
- Multiplex PCR
- Nested PCR
- Real-Time PCR

Detection Limit Range:
$0.1\text{-}3.2\times10^{8}$ spores/g soil

Detection Dependent:
Extraction efficiency, Type of Soil, Stains of *B.anthracis*

Most sensitive method:
PCR-ELISA, Nested PCR, PCR

Least sensitive method:
Biosensor Assay

Environmental Detection Limit of *Bacillus anthracis*

Air

Number of Spores Spiked

Air Sampler
- Flow rate: 15-150 L/min

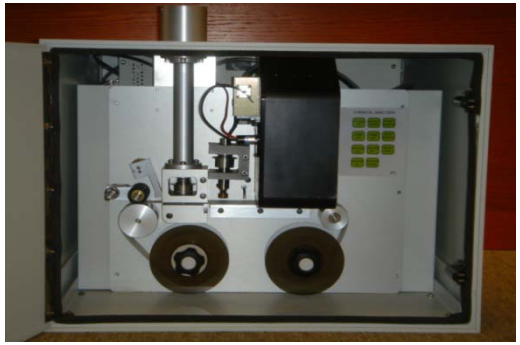
Extraction Method
- Thermal lysis

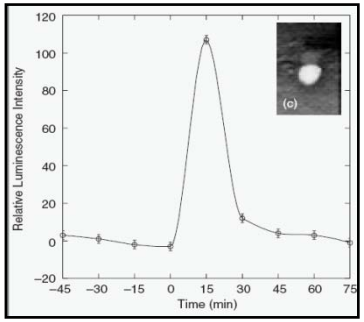
Detection Method
- ELISA-biochip system
- Lifetime-gated fluorimeter (ASD)

Detection Limit Range:
17-50 spores/L

Detection Methods:
ELISA-biochip System
Lifetime-gated Fluorimeter (ASD)

Detection Dependent:
Efficiency of air sampler





Environmental Detection Limit of *Bacillus anthracis*

Water

Number of Spores Spiked

Volume of Water
(0.1-10L)

Water Type
- Tap
- Source

Water Location
- Patuxent River, MD
- Chesapeake Bay, MD
- Beltsvile, MD
- Phoenix, AZ

Extraction Method
- 1,2,3 RAPID DNA extraction kit
- Qiagen DNeasy Tissue kit
- No extraction-filter placed on SRBC plate

Detection Method
- Cultivation Assay
- PCR
- Nested PCR

Sample Volume:
100 mL-10L

Detection Limit for Source:
Overgrowth-Cultivation
26 spores/mL-PCR

Detection Limit for Tap:
10 spores/10L-Cultivation
534 spores/L-PCR

Environmental Detection Limit of *Bacillus anthracis*

Fomites

Number of Spores Spiked

Surface Seeding
- Dry aerosol
- Inoculated spore solution

Fomite Area
($10cm^2$-$1m^2$)

Fomite Type
- Stainless steel
- Painted wallboard

Sampling Method
- Biological Sampling Kit (BiSKit)
- Cotton swab
- Macrofoam swab
- Polyester swab
- Polyester-rayon blend gauze wipe
- Rayon swab

Extraction Method
- Sonication and heat treatment
- Compressed foam
- Vortex in 5mL PBST for 2mins at 10s intervals

Detection Method
- Cultivation
  - Brain Heart Infusion Agar
  - Sheep Blood Agar
  - Trypticase Soy Agar
  - Trypticase Soy Agar w/ 5% Sheep Blood

Detection Limit Range:
10-100 CFU/cm^2

Method of Detection:
Cultivation

Recovery Efficiency:
11-44%

Extraction Efficiency:
93%

Detection Dependent:
Parameters affecting recovery

Quantifying Limits of Risk Estimates

Exponential Model

$$P(d) = 1 - e^{-kd}$$

P(*d*)= probability of death (when untreated)

d= average dose administered to the population

k= the probability that one organism will survive to initiate the response

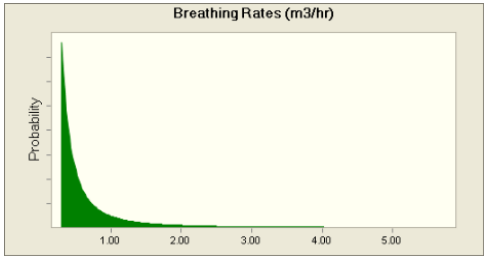
Dose

$$d = C_{air} \cdot R \cdot t$$

C_{air}= number of spores per cubic meter of air

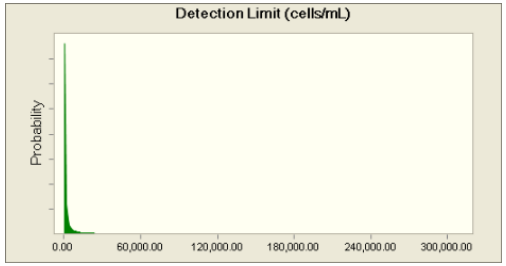
R= breathing rate (m^3/hr)

t= exposure time (hr)

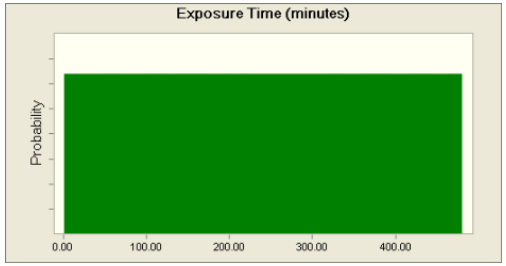


Breathing Rate
Pareto Distribution

(Short-term for adults (18+), both sexes, rest to moderate activity)

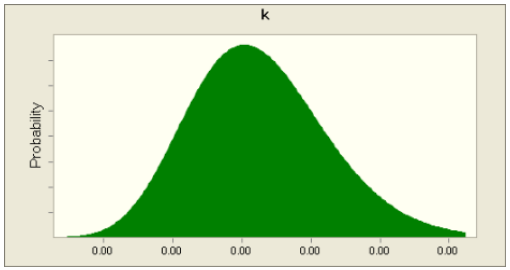


Detection Limit
Lognormal Distribution



Exposure Time
Uniform Distribution

(1 minute – 8 hours)



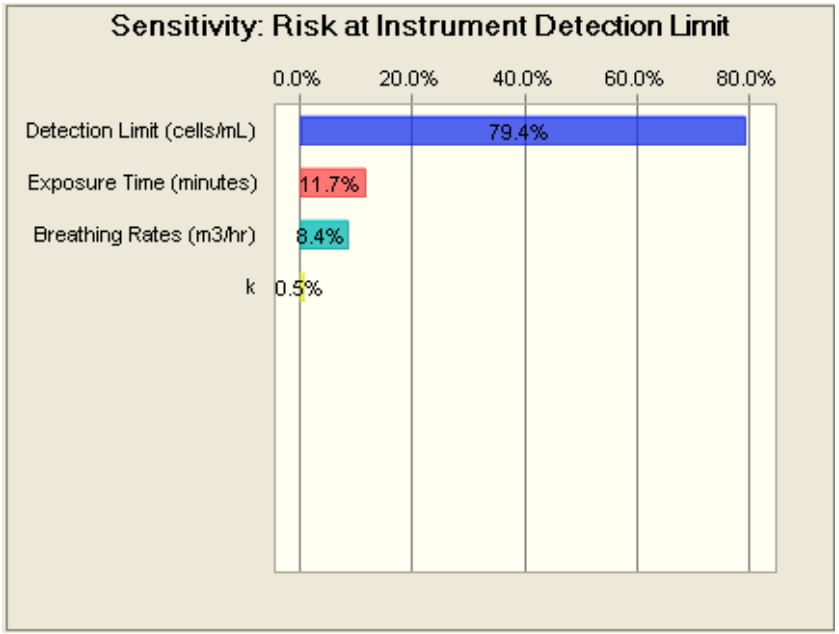
k
Gamma Distribution

(pooled guinea pig and rhesus monkey- 10,000 bootstrap reps)

Quantifying Limits of Risk Estimates

Risk Scenario	Analyzed Detection Limit	Median
Real-time PCR, Median Instrument Detection Limit	428.75 cells/mL	0.0063
Real-time PCR, Instrument Detection Limit	10-34,300 cells/mL	0.0062
Air, Lower Environmental Detection Limit	17,000 spores/m^3	0.221
Air, Upper Environmental Detection Limit	50,000 spores/m^3	0.520
Air, Environmental Detection Limit	17,000-50,000 spores/m^3	0.320

- **Log transformed PCR and real-time PCR**
  - Normally Distributed - Lilliefors Test (p= 0.65 PCR, p=0.78 real-time PCR)
  - Not Significantly Different - ANOVA (p= 0.94)
- **Assumption:** No loss in initial concentration during sample collection and processing
- **Estimated Risk:** 1 out of 160 people



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  - Sampling Techniques
  - Surface Area and Drying Time
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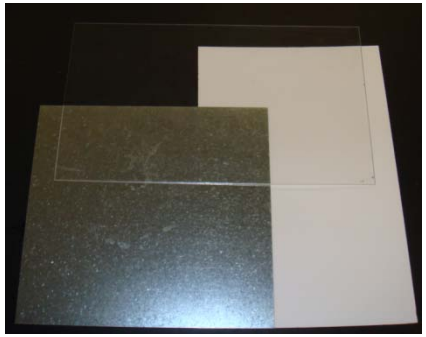
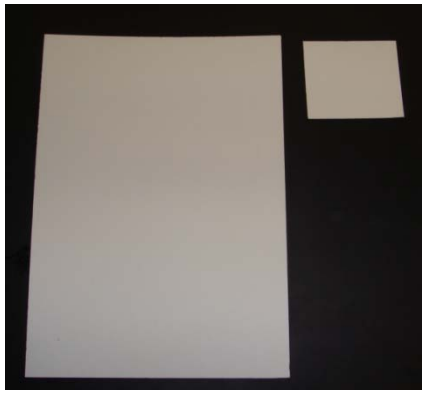
- **Conclusions**

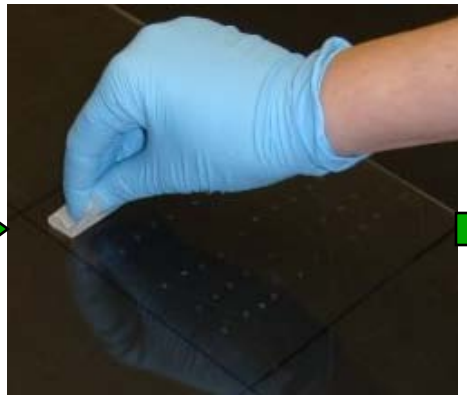
Fomite Recovery Studies at Low Concentrations

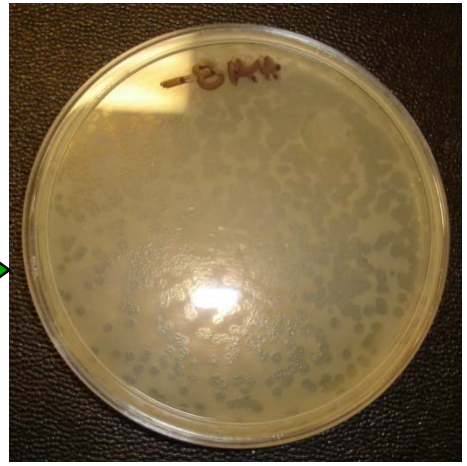
Agents	Bacteriophage P22
Fomites	Plastic, Stainless Steel, Laminar
Fomite Area	100 cm^2, 1000 cm^2
Sample Concentration	0.5 – 55 PFU/cm^2
Application Medium	Phosphate Buffered Saline Tween-80 (PBST), Trypticase Soy Broth (TSB), Water
Sampling Method	Pre-moistened Wipe, Kimwipe, Cotton Swab
Application Method	50 drops of 1µL
Time of Recovery	0 min and x (variable) min (dry)





Sampling Method





Percent Recovery

$$\%R = \frac{N_{assay}(D)}{N_{inoculated}} \times 100$$

% R = the recovery from fomite

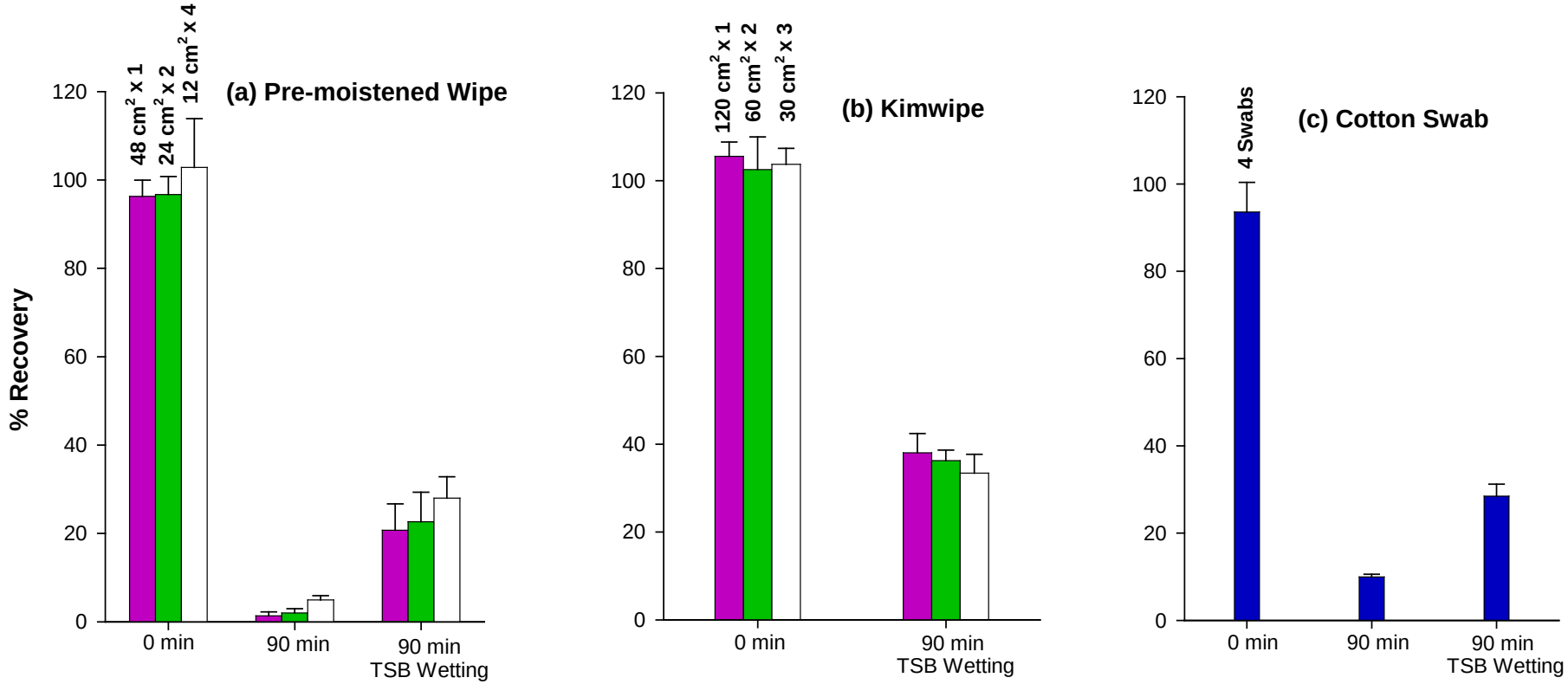
N_{assay} = the number of plaques on the agar plate

D = dilution factor (total volume of the extraction volume divided by the volume of sample assayed)

$N_{inoculated}$ = the number of P22 inoculated on the fomite

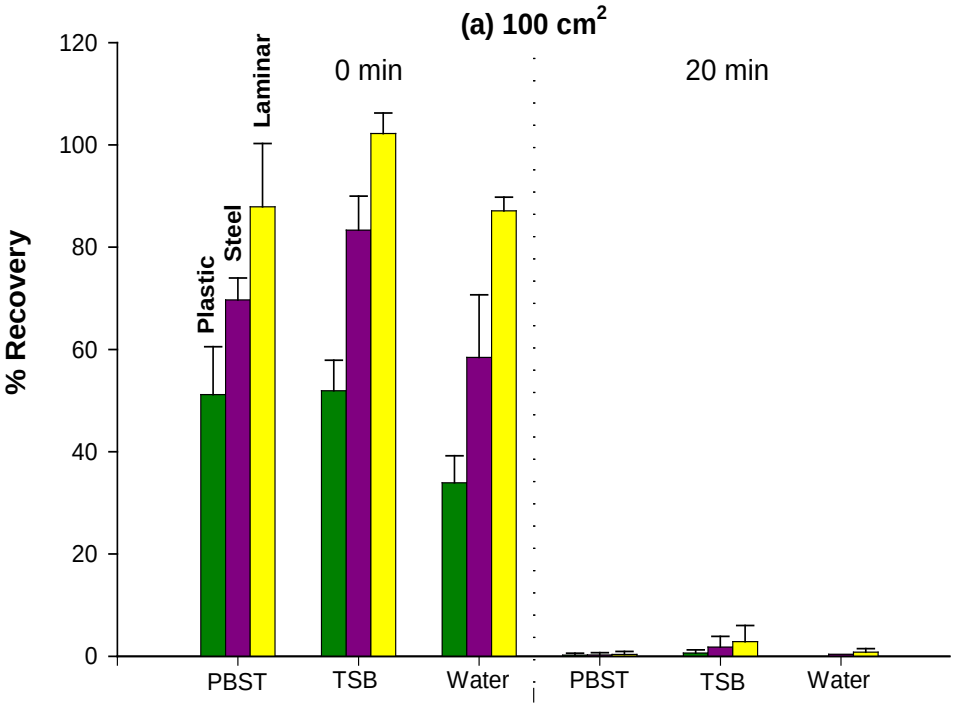
Sampling Technique

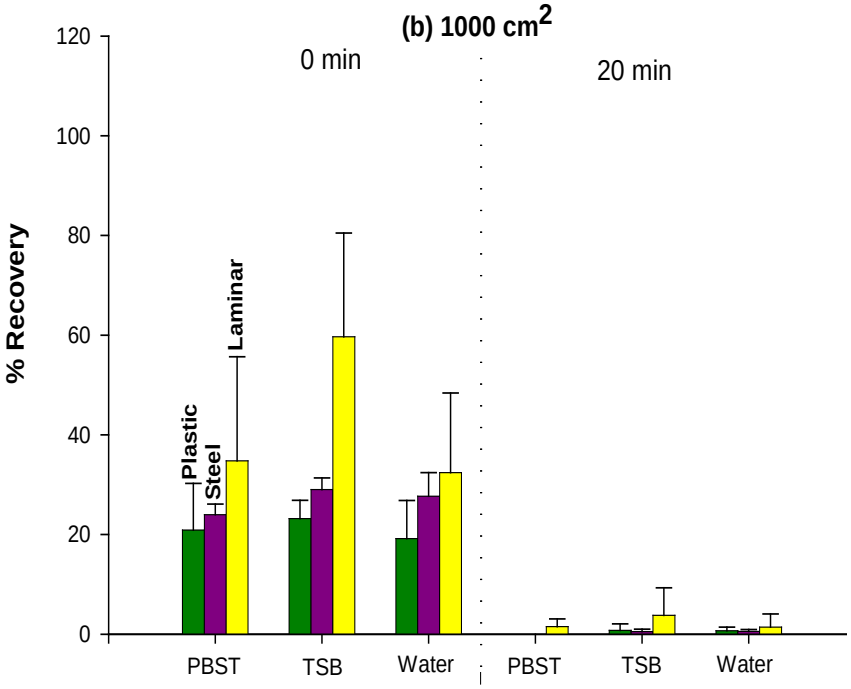
Fomite	Plastic
Fomite area	100 cm^2
Application medium	TSB
Application method	50 drops of 5µL
Average PFU/cm^2	**52**
Relative Humidity	40 %
Temperature	20.9 ºC



Surface Area and Drying Time

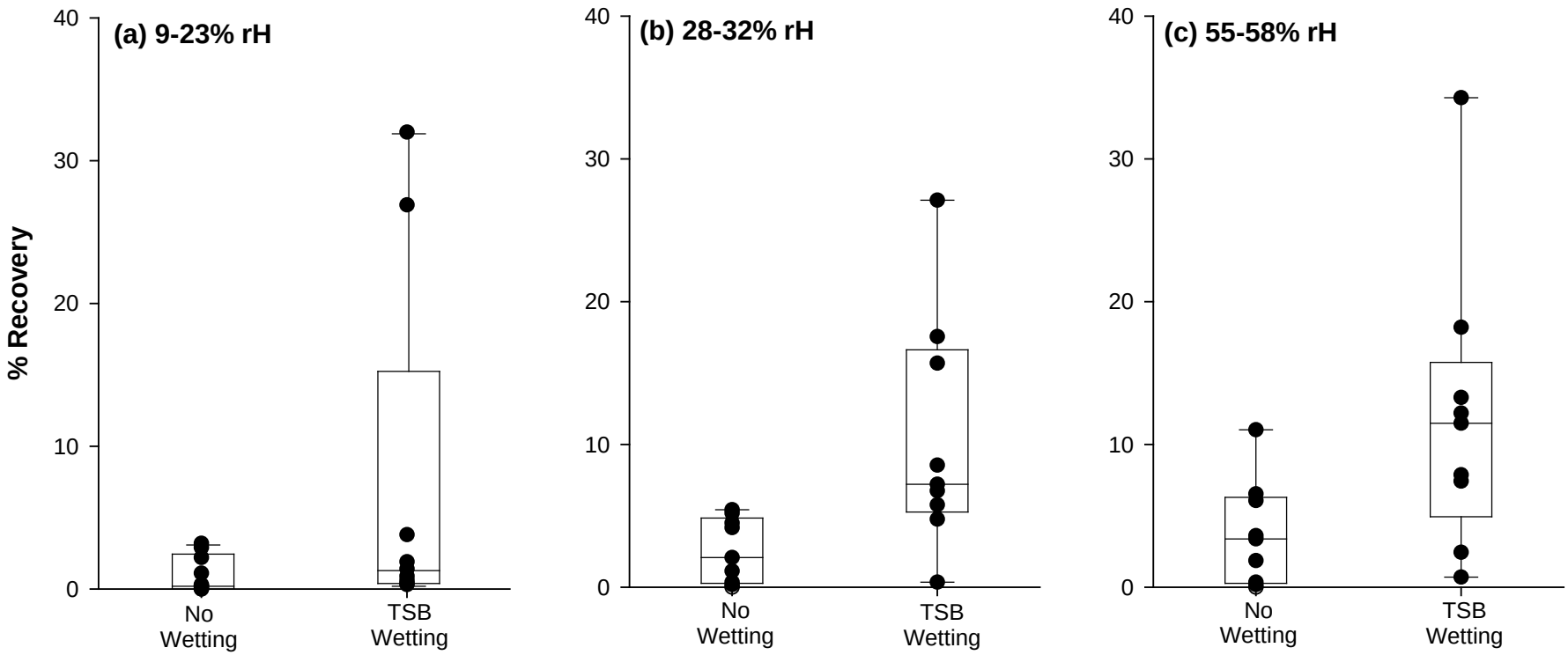
Fomite	Plastic, Steel, Laminar
Application medium	PBST, TSB, Water
Application method	50 drops of 1 µL
Average PFU/cm^2	4.36 1.93 (100 cm^2),
	0.44 0.19 (1000 cm^2)
Relative Humidity	20.6 12.7 %
Temperature	20.8 0.12 ^{o}C

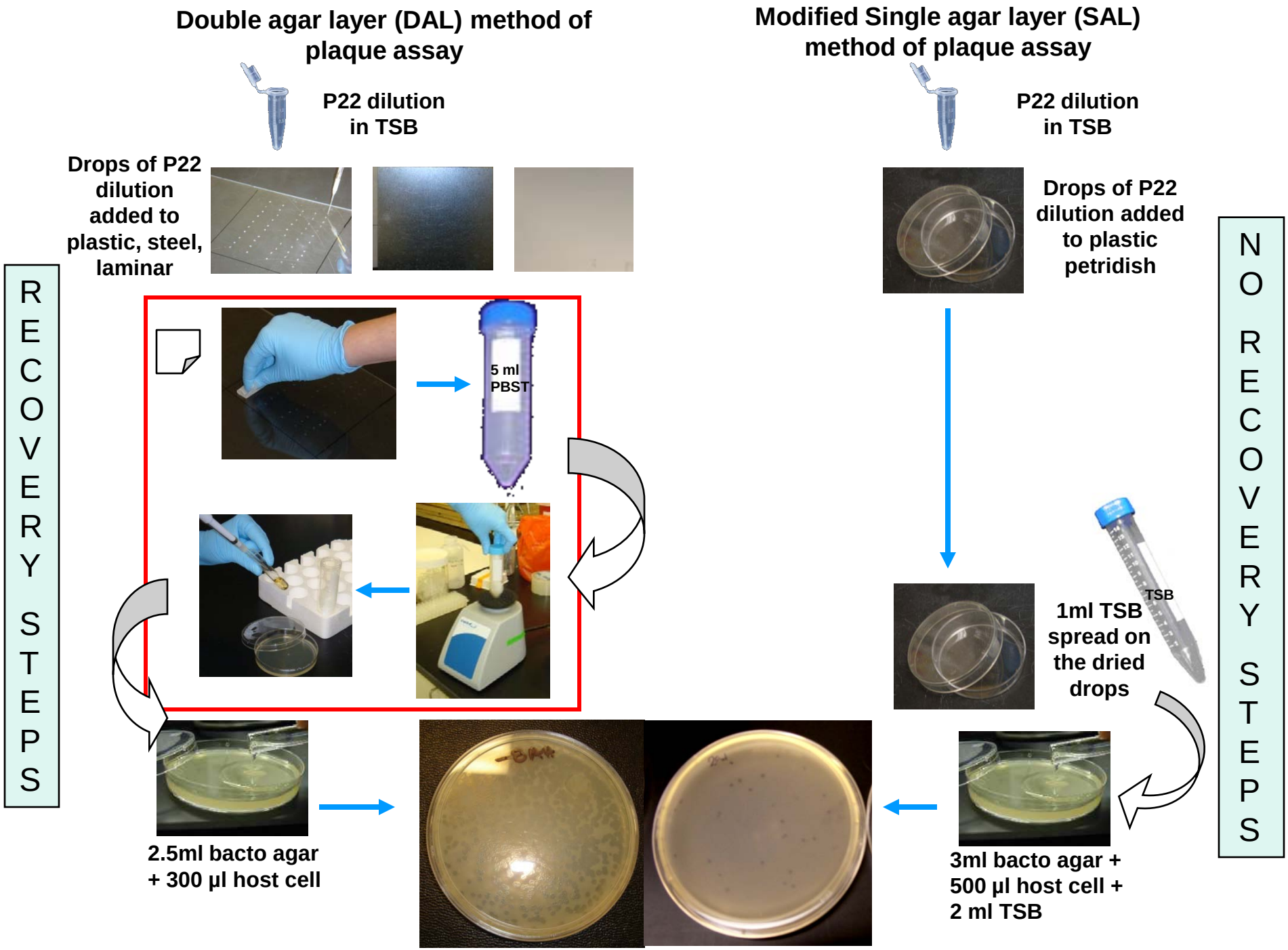




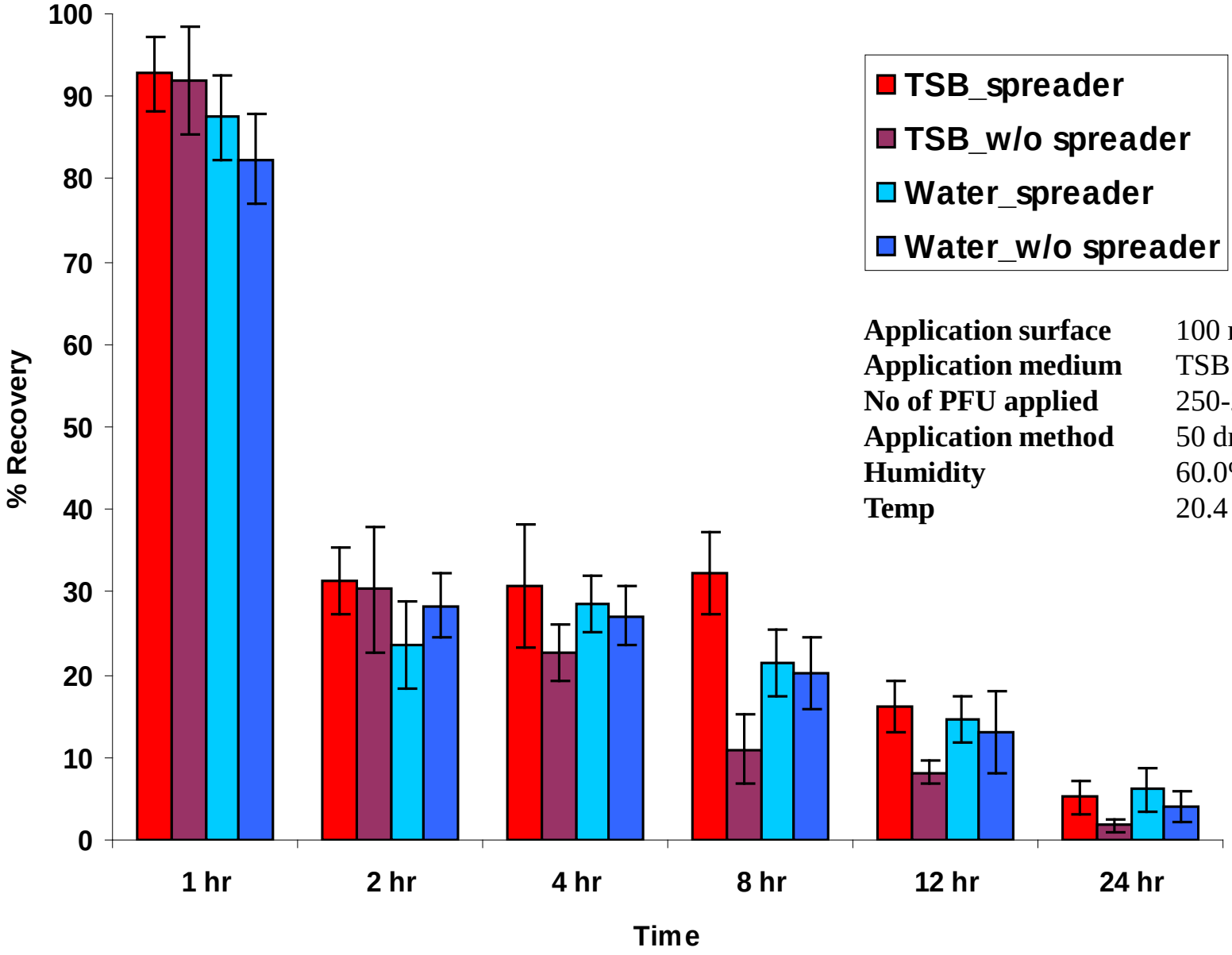
Impact of Wetting Agent at Varying Relative Humidity

Fomite	Plastic, Steel, Laminar
Fomite area	100 cm^2
Application medium	PBST, TSB, Water
Application method	50 drops of 1 µL
Average PFU/cm2	3.5 1.05
Temperature	21.1 0.56 ^oC




Double agar layer (DAL) method of plaque assay
P22 dilution in TSB
Drops of P22 dilution added to plastic, steel, laminar
5 ml PBST
RECOVERY STEPS
2.5ml bacto agar + 300 µl host cell
Modified Single agar layer (SAL) method of plaque assay
P22 dilution in TSB
Drops of P22 dilution added to plastic petridish
TSB
1ml TSB spread on the dried drops
3ml bacto agar + 500 µl host cell + 2 ml TSB
NO RECOVERY STEPS

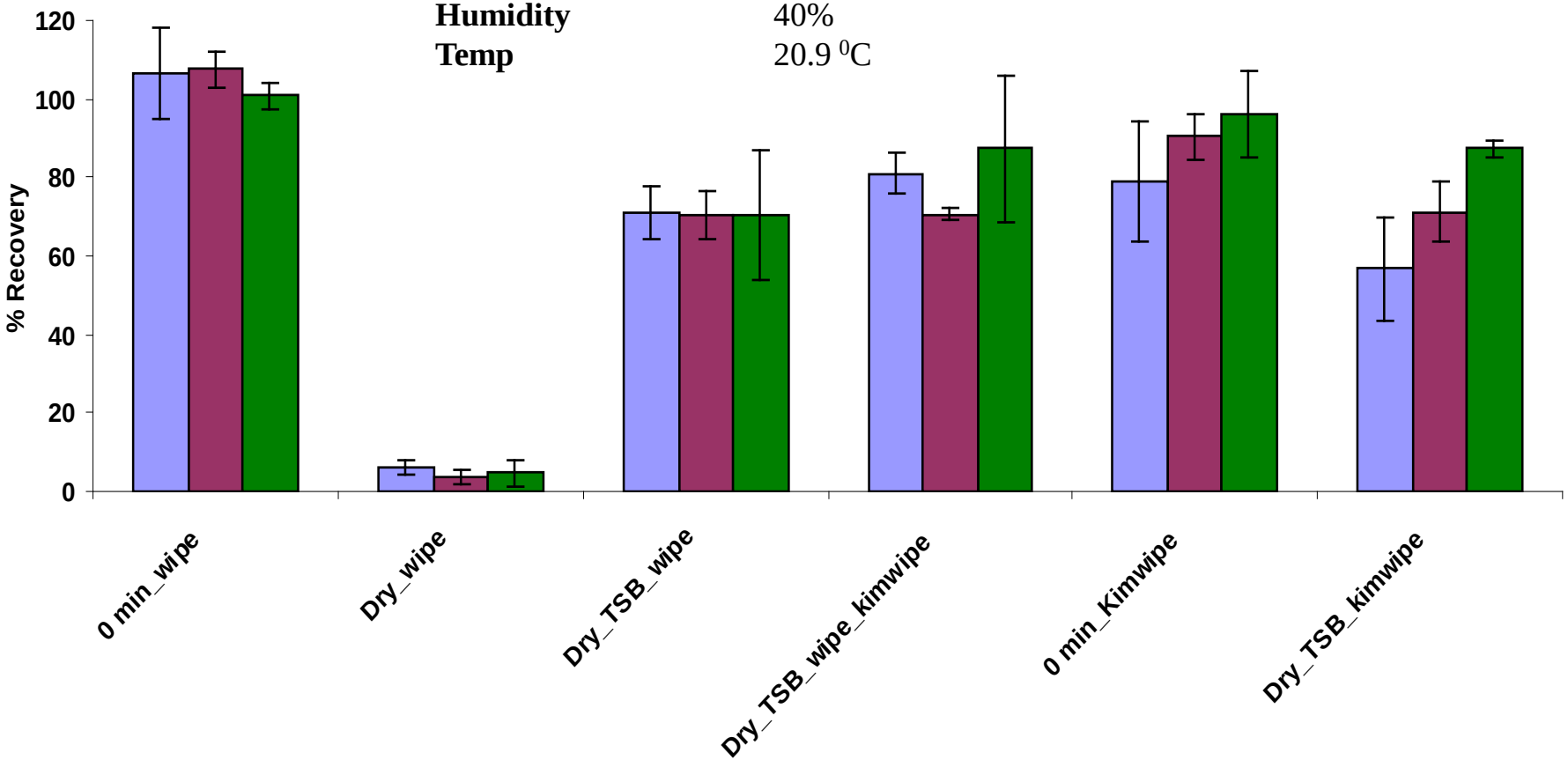
Temporal Recovery of P22 from Plastic Petri-dishes using Modified Single Agar Layer (SAL) Method



Application surface	100 mm petridish (PL)
Application medium	TSB / Water
No of PFU applied	250-275
Application method	50 drops of 5 µL
Humidity	60.0%-57.8%
Temp	20.4 ºC- 20.8 ºC

Recovery of *B. thuringiensis* Spores from Fomites

Fomite	Plastic, Steel, Laminar
Fomite area	100 cm^2
Application method	50 drops of 5µL
CFU/cm^2	9.0
Extraction medium	PBST (5 ml)
Humidity	40%
Temp	20.9 ^{0}C



Conclusions

- Environmental detection limit is critical for risk analysis and studies related to EDL are not many.
- Recovery methods at very low concentrations and large surface area need to be improved for estimates of risk at the lower limit.
- Decay (and not recovery) was a major reason for poor recovery for P22 under the conditions tested.

Acknowledgements

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