Advances in Applied Research

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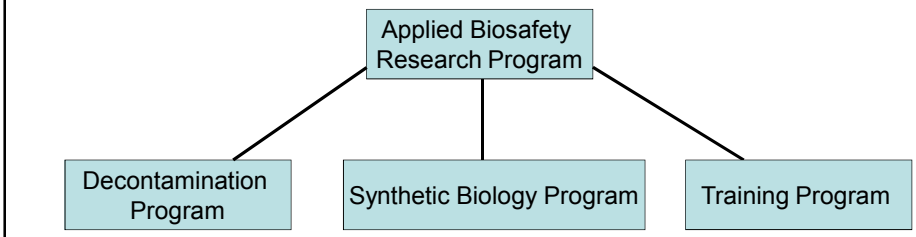
Applied Biosafety Research Program 'ABRP'

- Our focus at ABRP is to promote the Science of Biosafety, Bio-containment, Applied Research, and Biosecurity
- Develop stronger National/International based knowledge on Applied Research and develop Training Programs to assist in Biosafety issues globally

Mandate

- Develop, Design, and Validate
  - Engineering practices related to containment laboratories
  - Develop standards for disinfectant practices in containment laboratories and field outbreaks
  - Inactivation protocols for inactivation of CL3/4 samples (DNA,RNA, and Protein)
  - Efficacy studies of commonly used disinfectants against infectious disease
  - Infectious Clone Systems, used as a genetically marked standard

Program Research Areas



- CL3/4 disinfectant efficacy and Inactivation of infectious agents
- Develop national standards for decontamination
- Efficacy studies of commonly used disinfectants and development of National Data base

- Generate traceable/ quantifiable standards for use in efficacy studies
- Develop genetic backbones for use in:
  - Pathogenic Studies
  - Develop new diagnostic tools

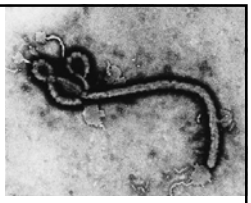
- Containment Training
- Biosafety Officer Training and First Responder Training
- Emergency Egress Training Program
- Applied Research techniques
- National/International Programs

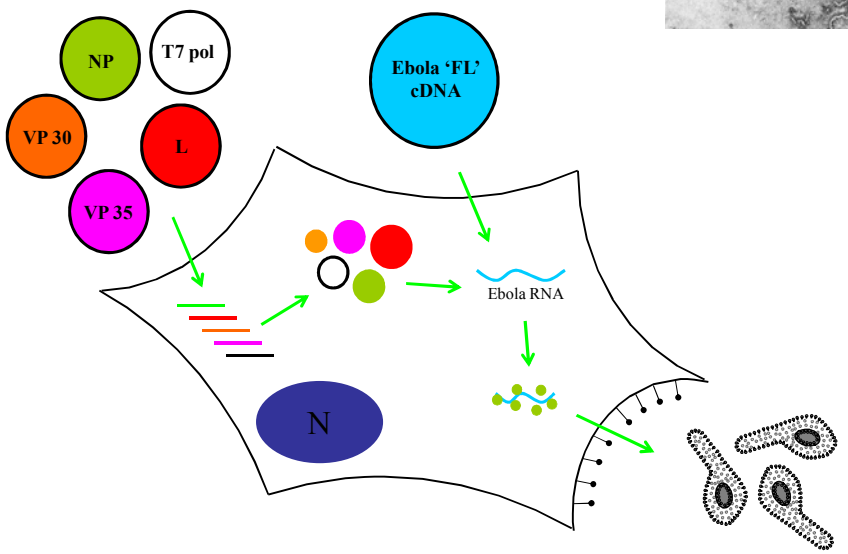
Synthetic Biology Program

Infectious Clone Systems

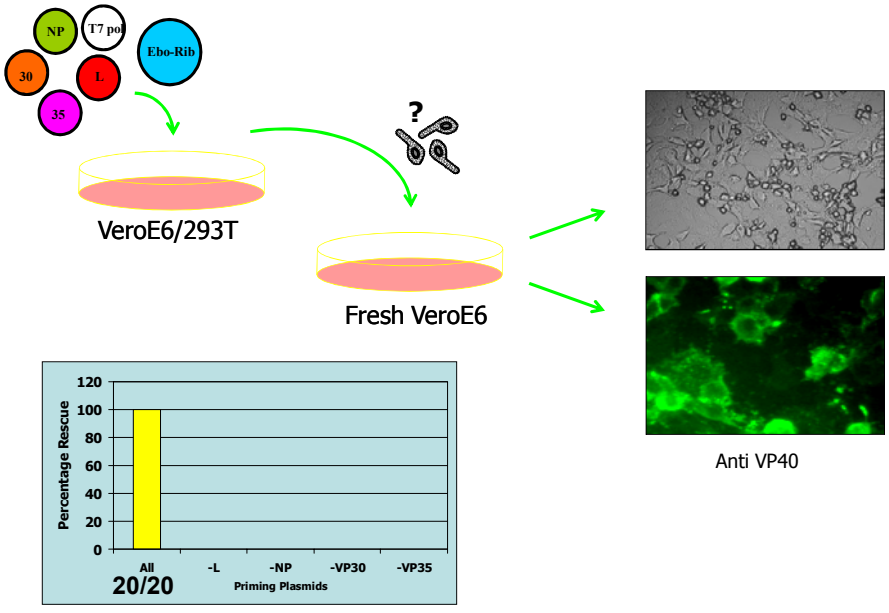
- Generation of florescent emitting organisms to be used as standard detection tools during validation experiments
- Genetic Backbones ‘vaccine development’
- Diagnostics
- Pathogenesis ‘site directed mutagenesis’

Ebola Reverse Genetics

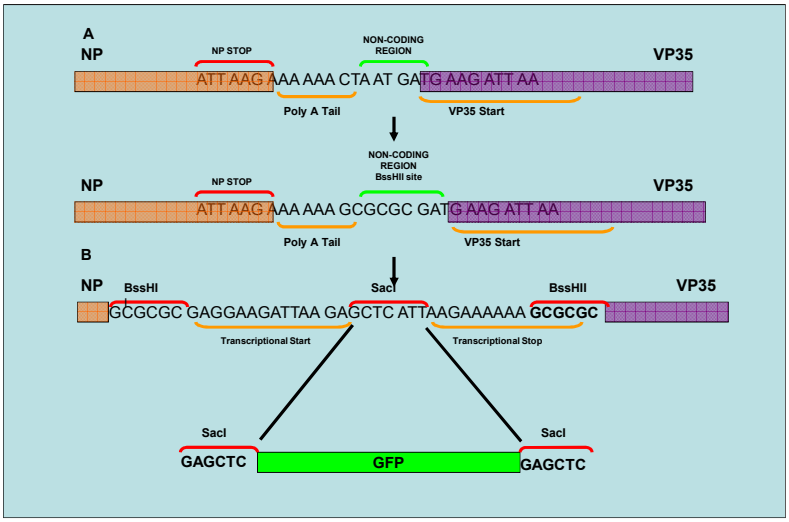




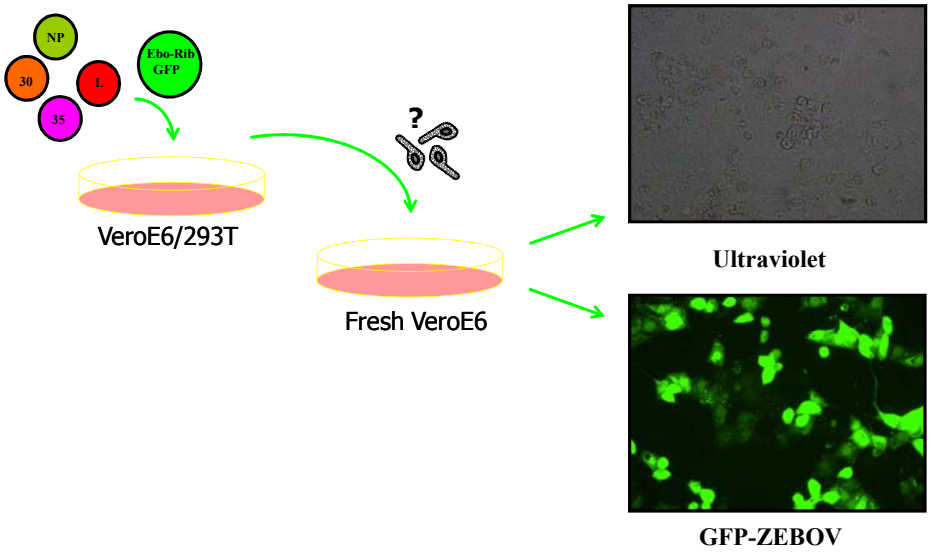
Ebola Virus Rescue



ZEBOV-GFP Construction



GFP-ZEBOV Virus Rescue



Other Genetic Clones Systems

- Generate traceable standards
- Develop vaccine backbones
- Pathogenic Studies
- Develop new diagnostic tools

- Avian Influenza-GFP
  - Green Fluorescent Fusion protein
  - Decontamination studies
  - Aerosolisation studies
- Kyasanur Forest Disease Virus (KFDV)
  - Flaviviridae 'infectious RNA'
  - Decontamination studies

Ebola Virus Synthetic Program

- Human Vaccine Development
- Guinea pig adapted virus production
  - INF response
  - Coagulation disorder
  - Gene/Protein Function
- Primate Vaccine Development

Decontamination Program

Where has all the data gone?

- Limited efficacy data for all viral families
- People assume data can be cross-references
  - Closely related viruses may differ in susceptibility
- Research conducted under laboratory conditions vary from laboratory to laboratory
  - Test conditions vary with virus, tissue culture type, and experimental design
- Very few research groups carrying out this type of research

(CL-4) Decontamination Project

'Experimental Goals'

- Determine the optimal concentration 'range' of disinfectants commonly used in containment laboratories (CL4)
- Test disinfectants include:
  - Microchem Plus - Virkon
  - Percept - Bleach
  - Ethanol
- Test organisms will include:
  - Filoviruses
    - Zaire Ebola virus 76' (Wild-type)
    - Zaire Ebola virus 'GFP' Recombinant virus

Experimental Procedures

Quantitative Carrier Tests
QCT-2

Inoculate carrier disk with soil load + virus

Carrier disk: Brushed stainless steel disk

Soil Load:
Mixture of BSA, Tryptone, and Mucin

↓

Add dilution series of disinfectant

Defined dilutions:
10%- 5%- 1%- 0.1%- 0.01%

↓

Defined contact time
1 min – 5 min – 10 min

Terminate test by adding neutralizer → Determine log reduction of virus '$TCID_{50}$'or 'FFU'

Disinfectant Range

ZEBOV-GFP

Disinfectant	(Min) Contact	(Min) Concentration	Log Reduction
MicroChem	10min	5%	7 logs
Virkon	10min	1%	7 logs
Bleach	5min	1%	7 logs
Percept	5min	5%	7 logs
Ethanol	10min	70%	7 logs
Ethanol	10min	95%	4 logs

Disinfectant Range

ZEBOV-GFP

Disinfectant	(Min) Contact	(Min) Concentration	Log Reduction
MicroChem	1min	5%	6 logs
Virkon	1min	1%	6 logs
Bleach	1min	1%	6 logs
Percept	1min	5%	5 logs
Ethanol	5min	70%	5 logs
Ethanol	10min	95%	4 logs

Validation of Extraction Protocols

Validation of CL4 Extraction Protocols

- Viral inactivation by nucleic acid extraction kits
- Tested Trizol LS, and AVL (RNA extraction kit) buffer against viral members from four genus types
  - Alphavirus – WEE
  - Bunyavirus – Rift Valley fever
  - Filovirus – Ebola (Zaire76) and Marburg (Musoke)
  - Flavivirus – West Nile

Validation of CL4 Extraction Protocols

- Both extraction methods were 100% successful in inactivation of any replicating viral species

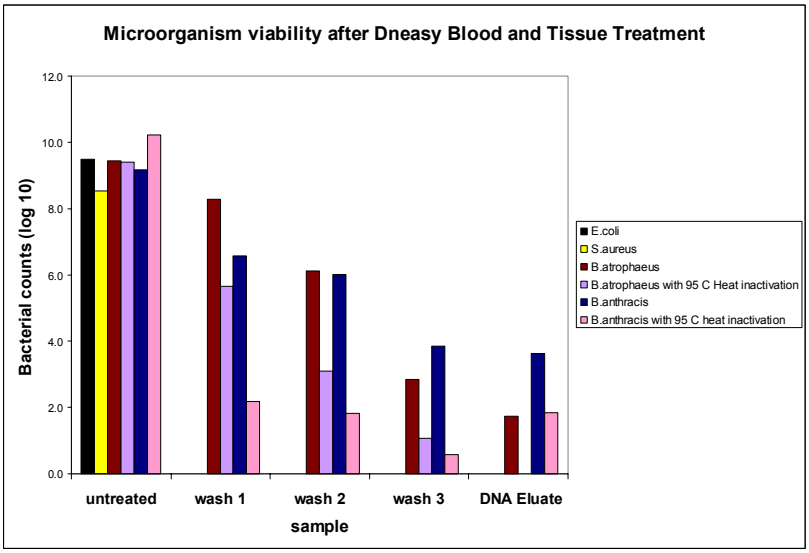
Rift Valley Fever (Bunyavirus)

	Viral load 10^7	10^6	10^5	10^4	10^3
Trizol LS	0	0	0	0	0
AVL buffer	0	0	0	0	0
DMEM media	$10^{5.8}$	$10^{5.0}$	$10^{4.2}$	$10^{2.3}$	$10^{1.7}$

Validation of CL3 Bacterial Extraction Protocols

- Bacterial/Spore inactivation by commercially available nucleic acid extraction kits
- Tested five extraction kits (Qiagen, Biorad)
- *B. anthracis*, *B. atropheaus*, and *S. aureus*

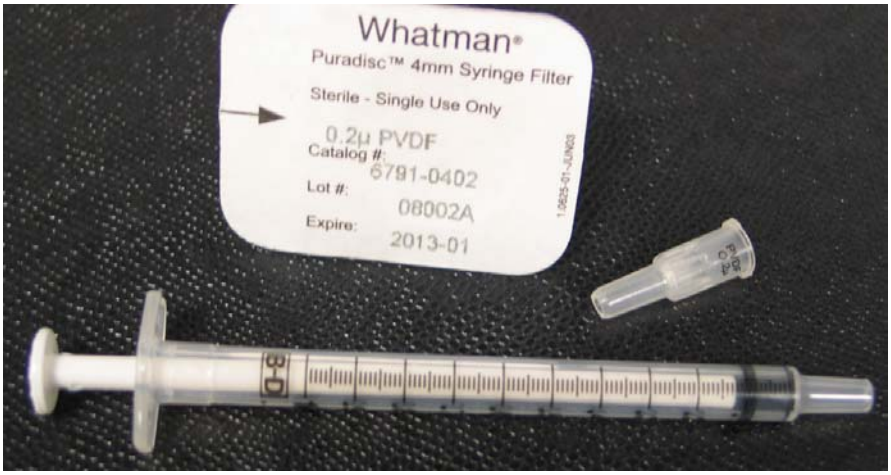
Validation of CL3 Bacterial Extraction Kit



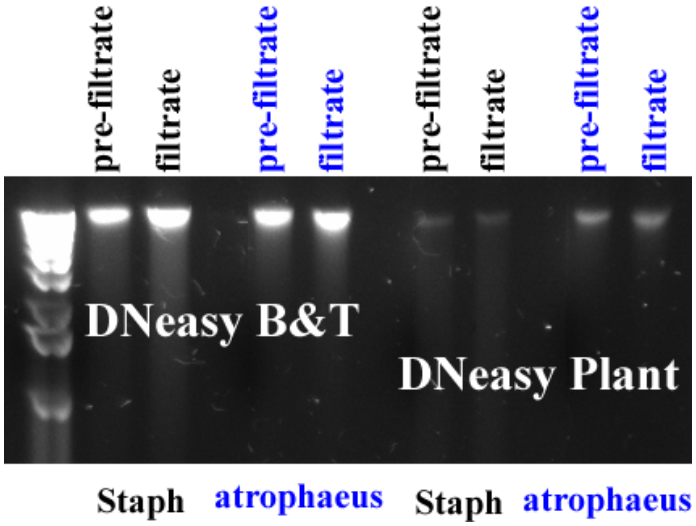
Conclusions

- Demonstrated the limitations of bacterial nucleotide extraction kits
- Demonstrate that heat treatment above 95^{o}C reduces the number of viable spores, and is necessary with spores formers
- Filtration is needed to fully remove any spores from the solution

Bacteriological Filter to Remove Bacteria



Filtration Has no Effect on DNA Extraction



Decontamination of Field Equipment and Containment Area

Decontamination of Field Equipment and Containment Area

- Utilizing gaseous decontamination methods
  - Assay the ability gaseous methods to decontaminate field equipment and field containment areas
  - Gaseous methods include:
    - Vaporous Hydrogen Peroxide (VHP)
    - Modified VHP (mVHP)
    - Gaseous Chlorine Dioxide (GCD)
  - VHP was dropped from the testing as soil loads would be encountered

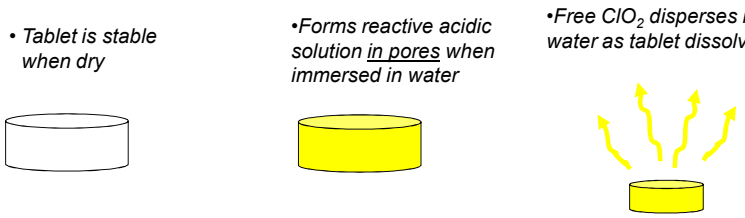
Decontamination of Field Equipment and Containment Area

- Chlorine Dioxide tablets and water to generate CD gas (no machine needed)
  - Tested this method during a decontamination of a 12x12 chicken barn
  - Chlorine Dioxide gas penetrated all areas inactivating all Bio-indicators
  - These methods will now be tested in a field situation
  - Promising results for decontamination of buildings after an natural outbreak or BT release

Uses in the Field

Methods of Generating Gaseous Chlorine Dioxide

- Tablets which rapidly generate activated ClO_2 solution and gas when immersed in water (Quip MB-10)
  - Typically 90% conversion of chlorite (ClO2-) to ClO_2
  - 8 to12 minutes depending upon size (1.5 – 6 grams)



Tablet GCD technologies can be applied to self-contained containment units

Space decon: Biological Safety Cabinet/Glove box

New Technology

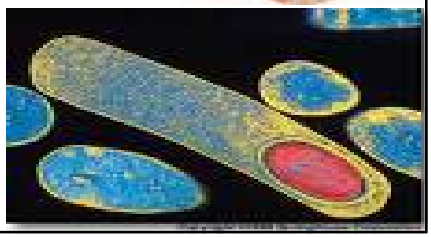
- Ozone mixed with hydrogen peroxide
  - Removes all spore formers in 25min after saturation
  - Ozone is removed with in 20min after exposure
  - No residual effects, no clean up
  - One problem….. Need of 80% humidity

Decontamination of Hospital Equipment and Containment Area

Decontamination of Hospital Equipment using a Bacteriophage

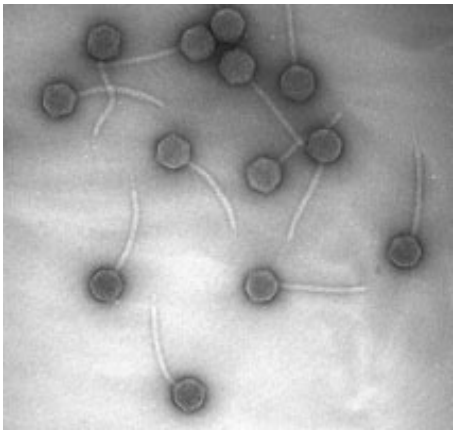
- Hospital acquisition of *C. difficile* is increasing
- *C. Difficile* is a spore forming enteric Bacillus
- Inproper sterilization of instruments can render than inoperable and costly to replace
- Decontamination of hospital rooms
  - Lots of different surfaces (metal, fabric, rubber etc)
  - Small cracks in surfaces make liquid penetration difficult

Decontamination of *Clostridium difficile* in a Hospital Environment



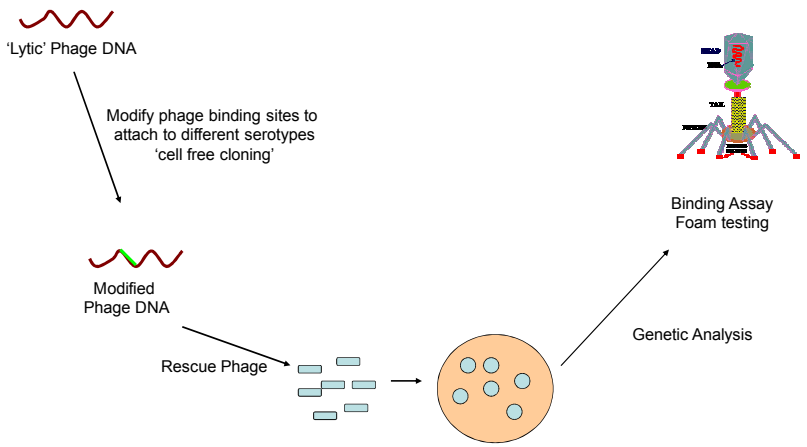
Bacteriophage ΦCD119 Lysin

- ΦCD119 lysogenic virus
- Only infects and lysis limited number of *C. difficile* strains
- Lysin from phage affects 50+ strains tested to date

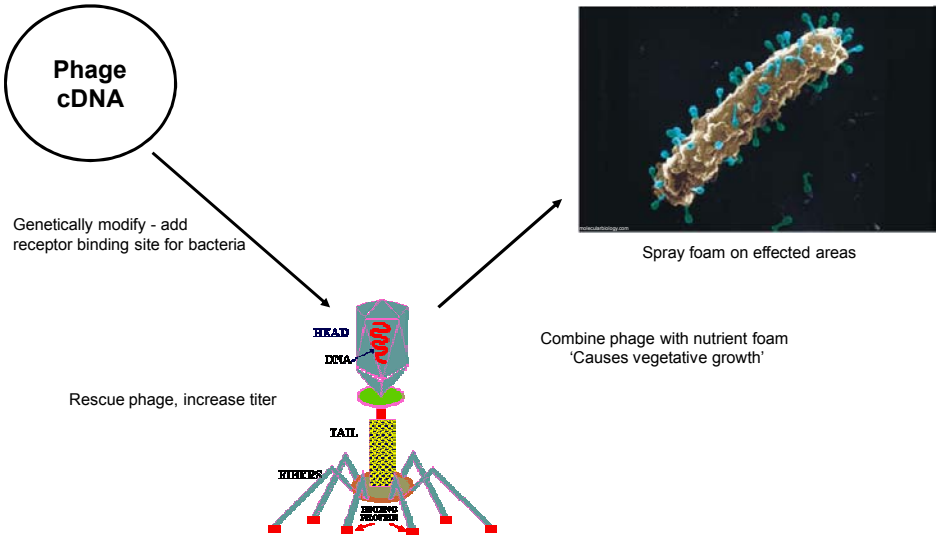


Phage Development

'The Process'

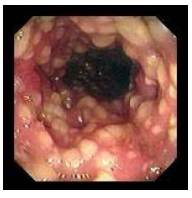


Development of Bacteria Inactivating Phage

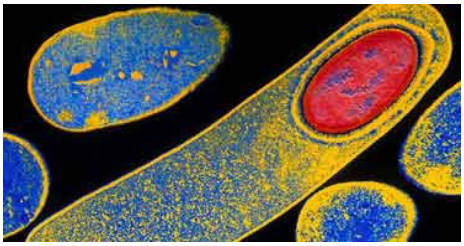


Application of Genetically Produced Bacteriophage

- Inactivate *B. anthracis* using lytic phage
  - DRDC used on military equipment
- Control *Clostridium difficile* in hospital environment
  - Many different surfaces (metal, fabric, rubber)
  - Small cracks in surfaces make liquid decontamination process difficult



Germination of Dormant *C. difficile* Spores



- Normal chemical treatment needed to kill spores may damage equipment and may be ineffective
- Vegetative bacteria susceptible to chemicals
- Bile Salt, taurocholate and Glycine to germinate spores

Decon Delivery System

- 15-30% fumed silicate (SiO_2)
- Stable (pH, Time, Oxidation etc.)
- Ease of application (paint sprayer)
- Foam has advantage of:
  - penetrating into cracks
  - remains wet for prolonged periods
  - able to stick to vertical and inverted surfaces
  - Dries and easily vacuumed up
  - Can be interchangeable with other decon agents

Putting it all together

- Produced a Foam based disinfectant that:
  - Is easy to apply
  - Contains spore germinating compounds
  - Contains Biologically active ΦCD119 lysin to rupture vegetative bacteria
  - All components are non toxic for general use

Project Review ABRP

Formaldehyde Biological Indicators

- Current indicators used at NML as well as across Canada
- Not for use with formaldehyde, common use is in autoclaves
- BI have been shown to produce false negatives due to formaldehyde retention

Testing for Residual Formaldehyde

Residual formaldehyde Concentration

- >50 BI with paper strips tested
  - Residual formaldehyde present in all concentrations range 1.3 to >45 mg/l

Conclusions

- Biological indicators composed of paper spore strips do absorb formaldehyde during decontamination events
- The concentration of formaldehyde found in the indicators inhibits the growth of spores

Conclusions

- Need to test a non-absorptive carrier such as stainless steel
  - Preliminary results show that stainless steel carriers do not absorb formaldehyde
  - Using these for BIs does not inhibit the growth of spores

Listeria decontamination efficacy using Maple Leaf decon agents

- Determine the efficacy of decon agents currently used at Maple Leaf as well as new products.
- Data has been complied and sent to ML for all decon agents.
  - Resulted in change of cleaning methodologies
  - Peer reviewed publication (Summer 2011)

Acknowledgements

- Special Pathogens Program
- University of Manitoba
  - Microbiology Department
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- International Center of Infectious Disease
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Questions?