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Canada 

Resistance Determination of a Respiratory Pathogenic Strain of *Bacillus cereus* to Liquid Disinfectants

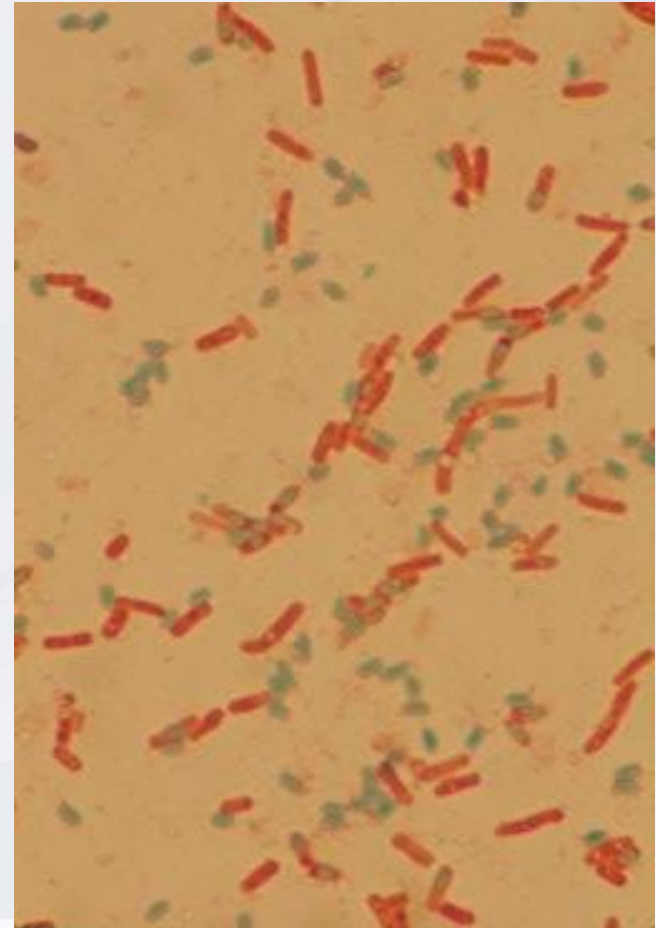
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3. Beijing Center for Disease Prevention and Control, Beijing, China

Bacillus cereus

- Rod shaped, gram positive bacteria
- Spore formers
- Ubiquitous in nature
- Some strains are opportunistic pathogens
- Serious disease in non-immunocompromised hosts is unusual
- Genetically closely related to *B. anthracis*

Spore Stain



Differences between *B. cereus* and *B. anthracis*

- *B. cereus*

- Hemolytic
- Motile
- No poly-D-glutamic acid capsule
- resistant to lysis by gamma-phage
- **Typically no plasmids**

- *B. anthracis*

- Non-hemolytic
- Non-motile
- poly-D-glutamic acid capsule
- Not resistant to lysis by gamma-phage
- **pX01 and pX02 plasmids**

***B. anthracis* plasmids**

- pX01: contains genes *lef*, *cya* and *pa*
 - *lef* – lethal factor
 - *cya* – edema factor
 - *pa* – protective antigen
- Altogether they cause edema and necrosis which is lethal
- pX02: contains genes for the poly-D-glutamic acid capsule

Background

- *Bacillus cereus* strains that caused fatal pneumonia in healthy humans
 - Resembled anthrax-inhalation
- *Bacillus cereus* G9241
 - Almost complete pX01 plasmid
 - pBC218 plasmid – different capsule

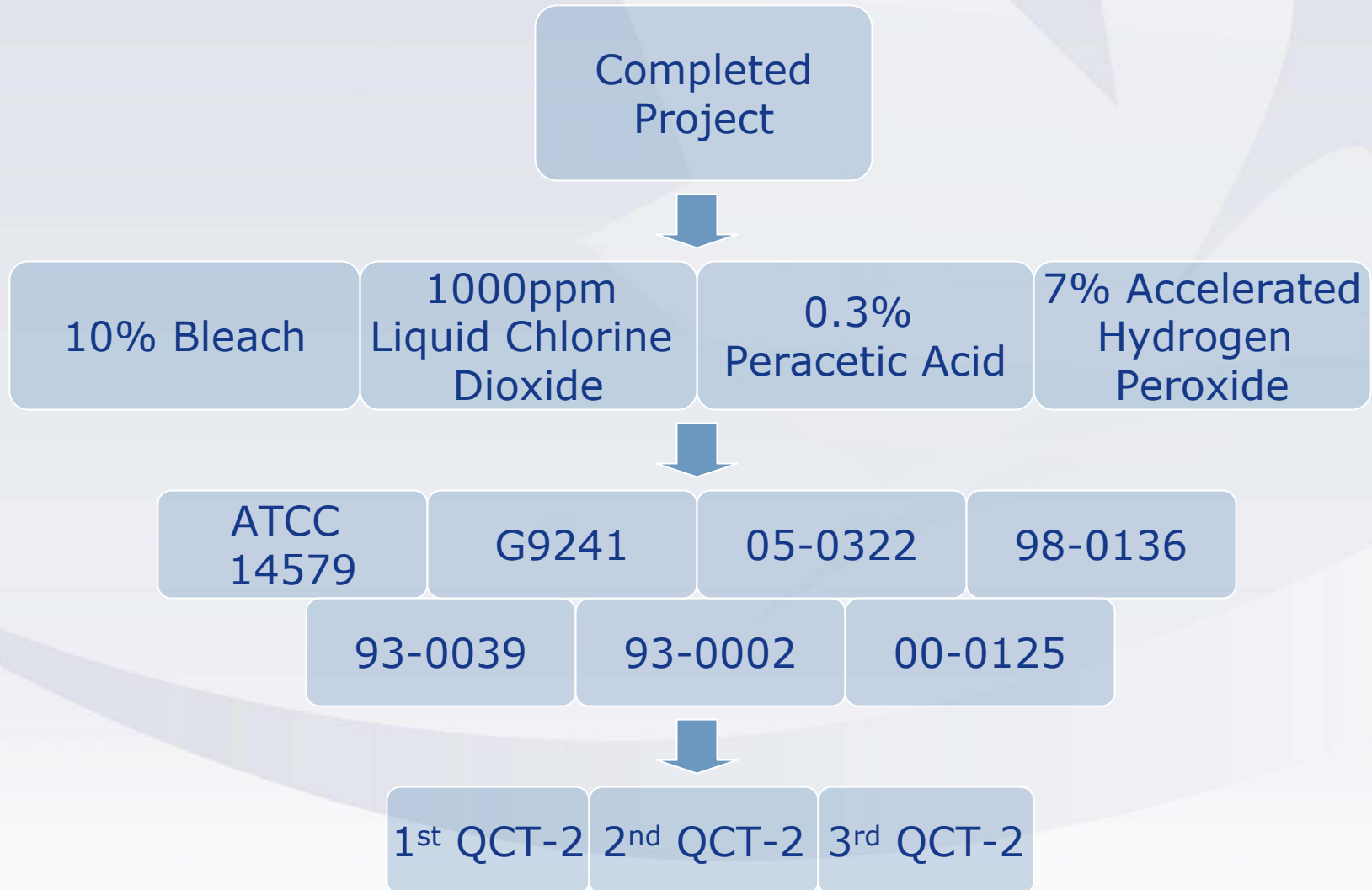
Background

- Previous unpublished studies have shown that G9241 has unusually high resistance to some liquid disinfectants compared to *B. anthracis*
- Potential to become a significant, emerging respiratory pathogen
 - Ability to produce anthrax toxins
 - Signs of higher resistance

Objective

- Determine if G9241 has a higher resistance to liquid disinfectants in comparison to other *B. cereus* strains
- Prevent transmission from occurring because of improper disinfection

Materials and Methods

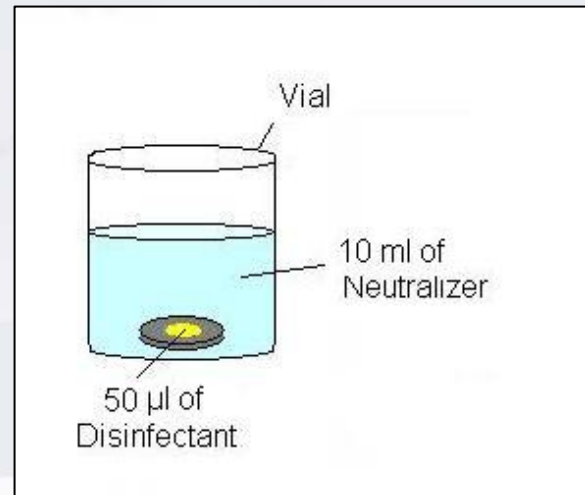
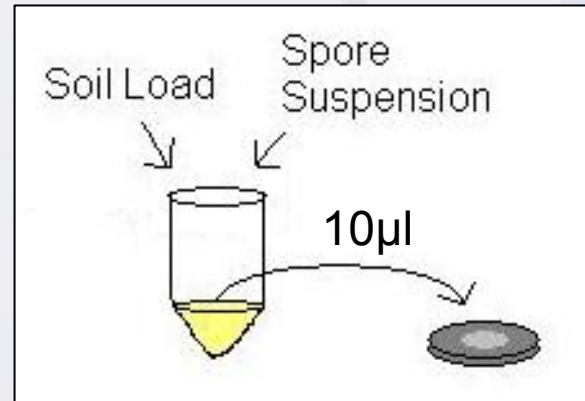


Quantitative Carrier Test-2

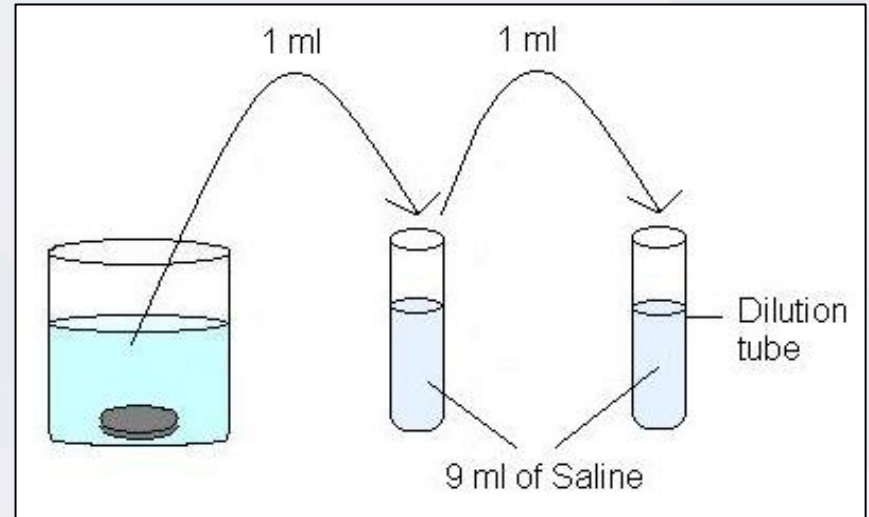
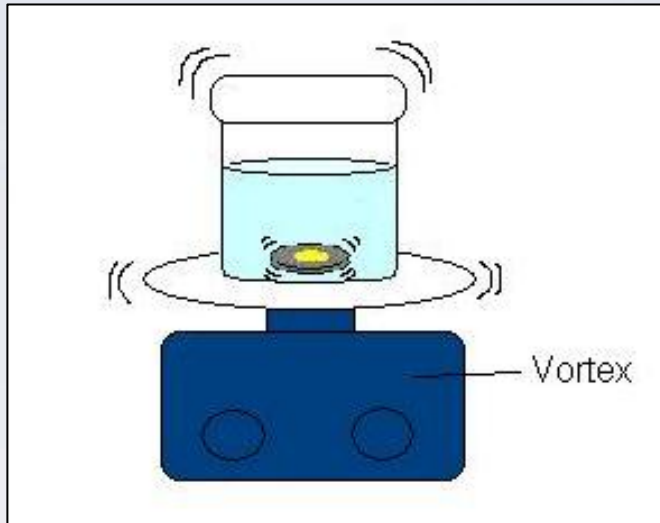
- The second tier of a quantitative carrier test (QCT-2), a standard of ASTM International
- One QCT-2 Test uses 9 stainless steel carriers
 - 3 control carriers
 - 2 carriers for 5 minutes
 - 2 carriers for 10 minutes
 - 2 carriers for 20 minutes

Quantitative Carrier Test-2

- Dry 10 μ l of a soil load and spore mixture onto a stainless steel carrier
- Add 50 μ l disinfectant
- Add 10ml neutralizer after contact time



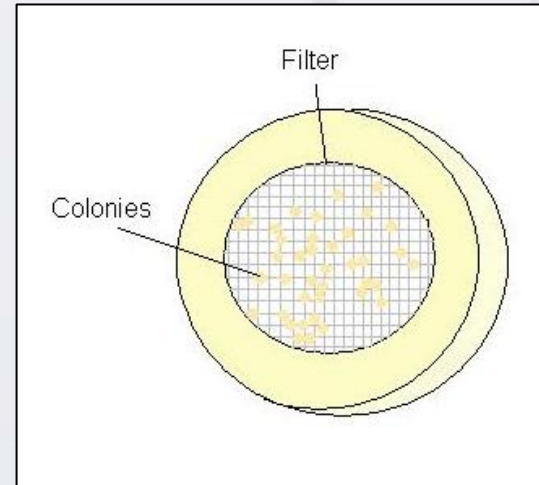
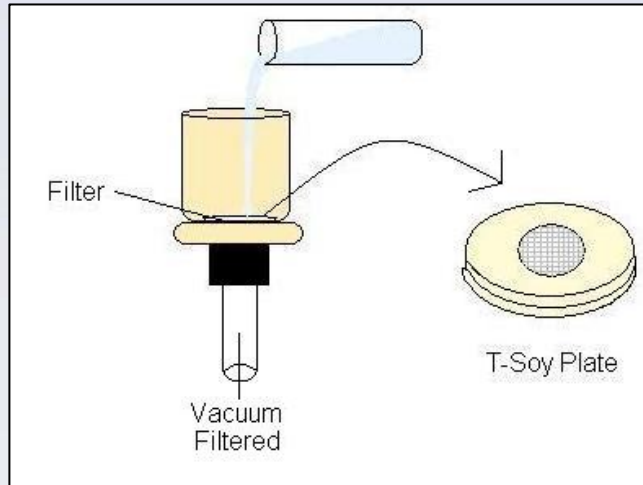
Quantitative Carrier Test-2



- Vortex for 1 minute

- Perform 10-fold dilutions

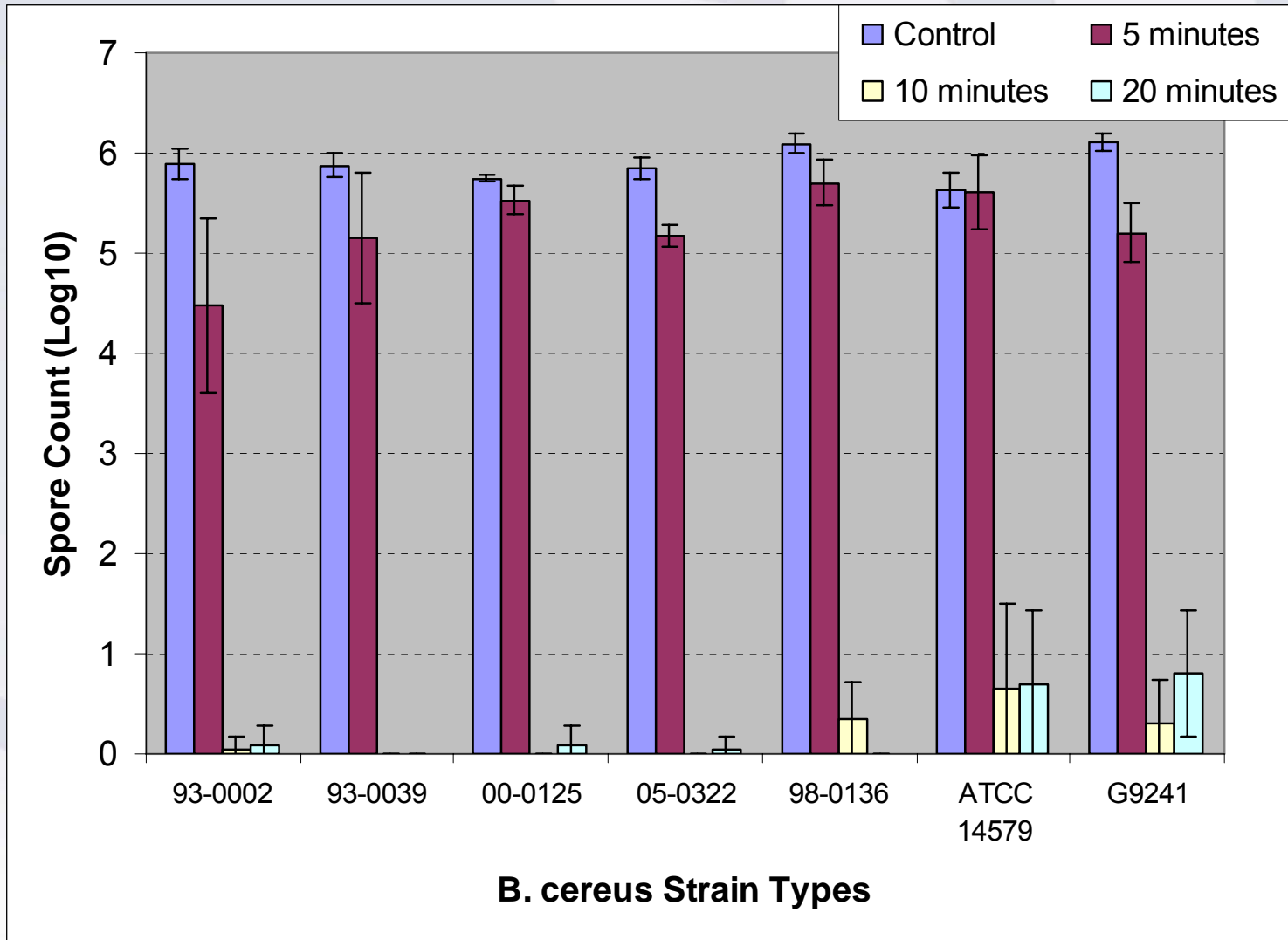
Quantitative Carrier Test-2



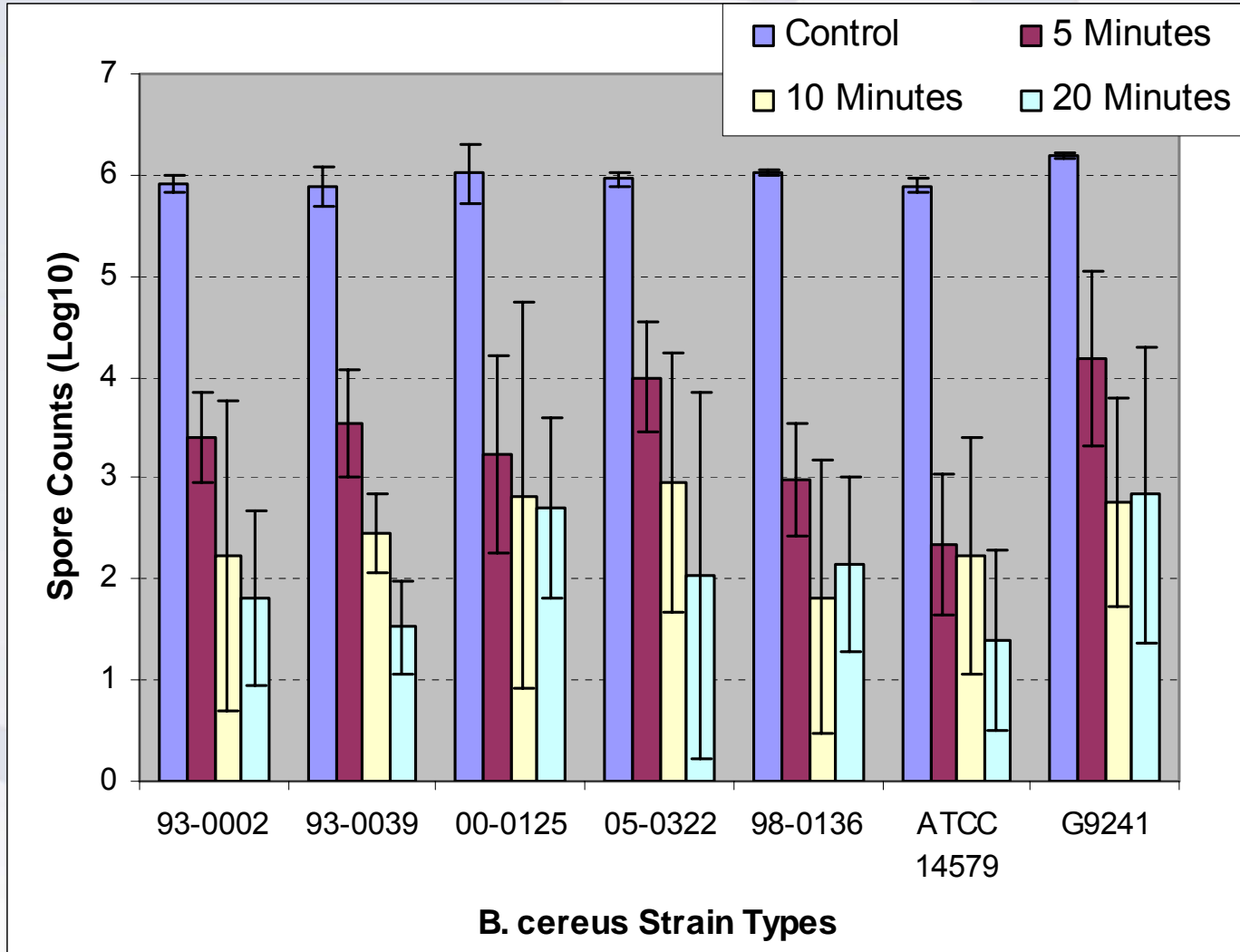
- Dilutions were vacuum filtered and plated on T-soy agar plates

- Plates were incubated and colony forming units were counted and recorded

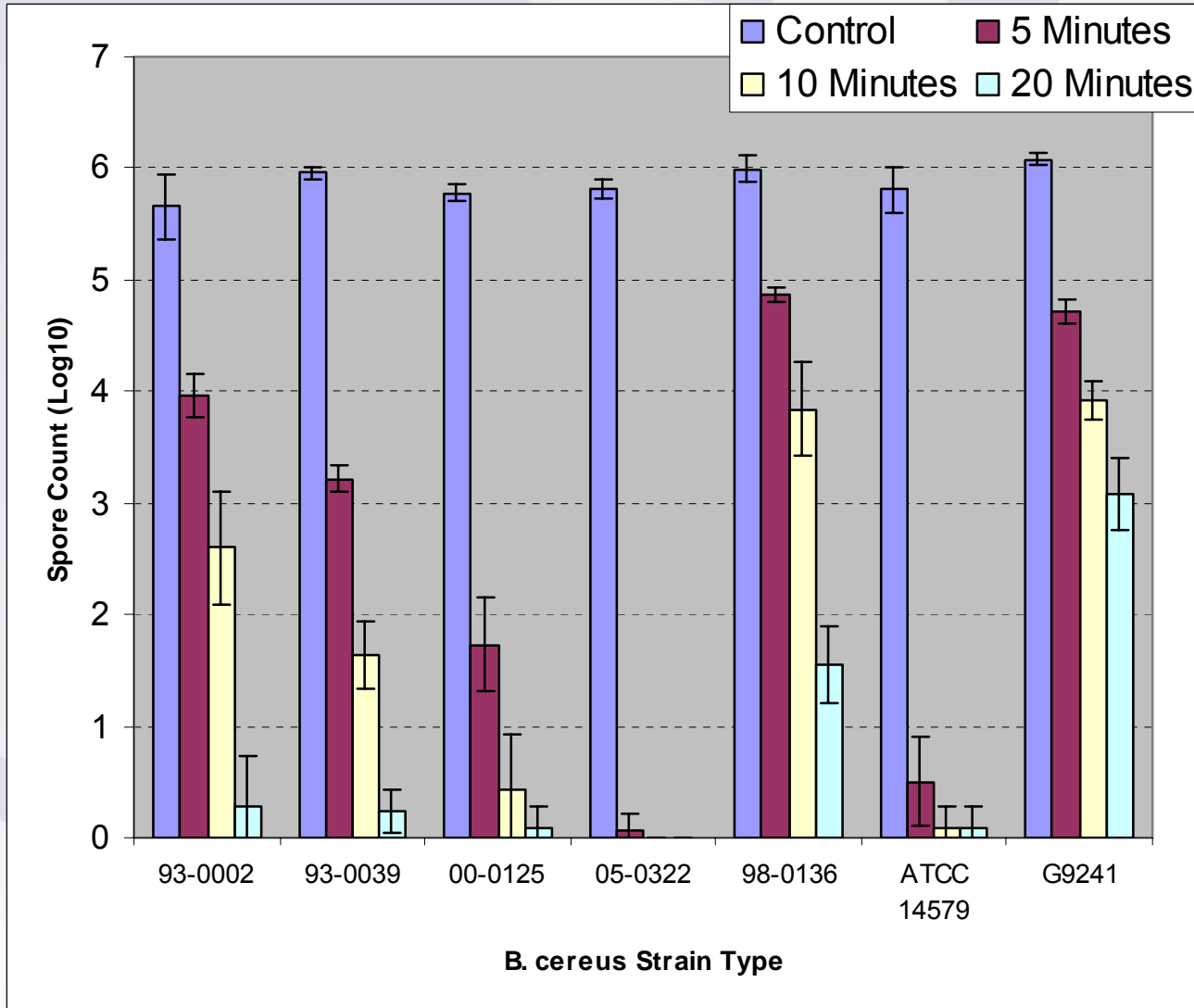
10% Bleach



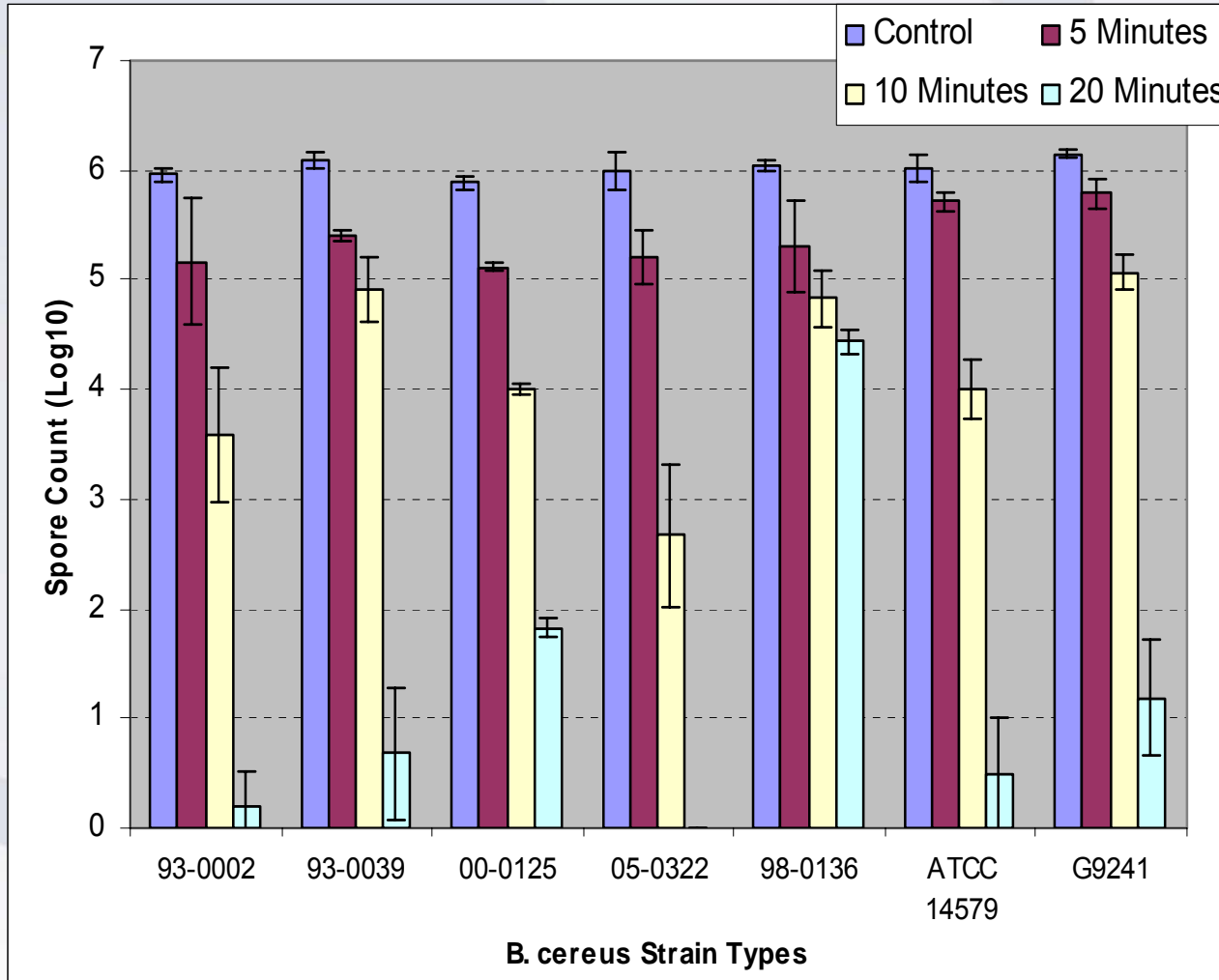
1000ppm Liquid Chlorine Dioxide



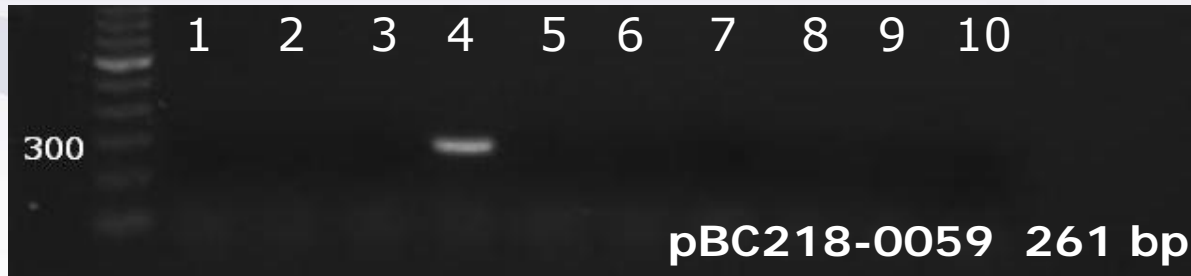
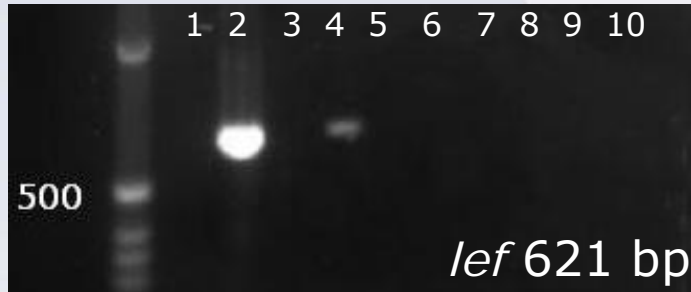
0.3% Peracetic Acid



7% Accelerated Hydrogen Peroxide



PCR Analysis



1. Water

B. anthracis

2. Sterne Strain

3. ATCC 4229

B. cereus

4. G9241

5. 00-0125

6. 05-0322

7. 93-0002

8. 93-0039

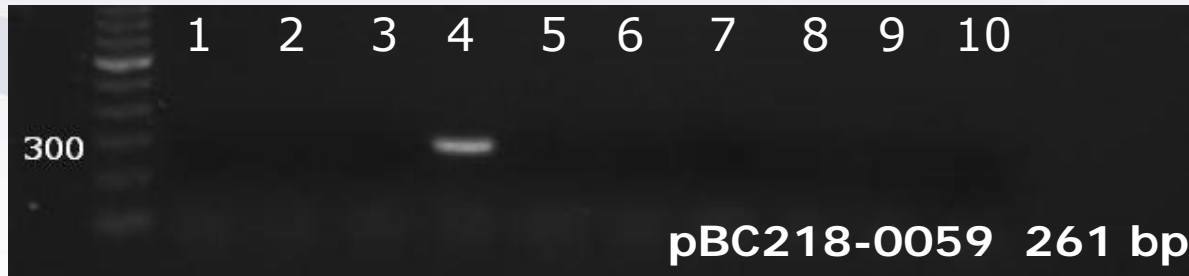
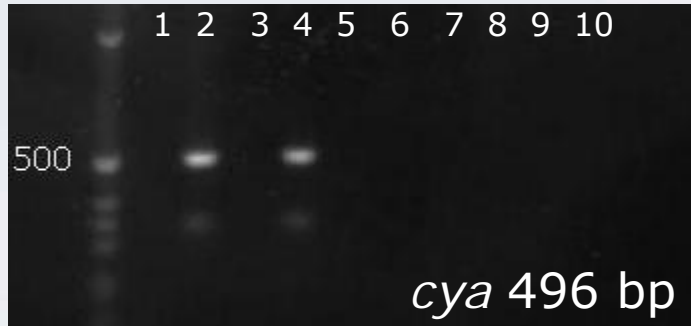
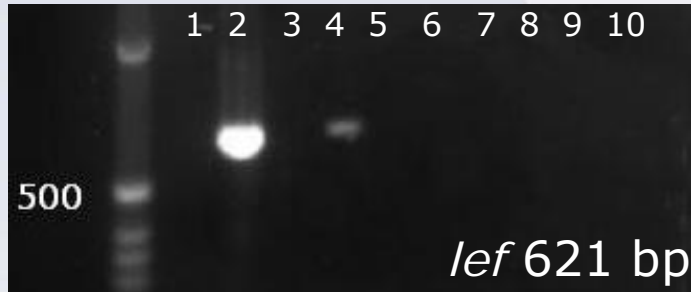
9. 98-0136

10. ATCC 14579

Conclusion

- *B. cereus* G9241 may be harder to disinfect using peracetic acid
- Most effective and consistent – 10% bleach
- Even closely related organisms can react differently to various disinfectants
- Facilities should test disinfectants first

PCR Analysis



1. Water

B. anthracis

2. Sterne Strain

3. ATCC 4229

B. cereus

4. G9241

5. 00-0125

6. 05-0322

7. 93-0002

8. 93-0039

9. 98-0136

10. ATCC 14579

DNA Extraction

- Even after DNA extraction, DNA was not taken out of CL3
- Are the reagents in the commercial kits enough to inactivate pathogens?

Survival of Bacterial Spores Following DNA Extraction Procedures

Cristal Cizik, Diane Gordon, Todd Cutts, Jay Krishnan, Steven Theriault

Applied Biosafety Research Program,
Public Health Agency of Canada, Winnipeg

Background

- **Nucleic acid preparations from RG3 and RG4 agents are extracted using commercially available kits**
- **These NA preparations are often dunked out of high containment labs for analysis in CL-2 labs.**
- **It's unclear if the extraction procedures inactivate the microbes**

Objective:

- **To determine if commercially available nucleic acid extraction kits have the ability to inactivate microorganisms**

Materials and Methods

•Microorganisms

S.aureus (25923)
B. atrophaeus (51189)
B. anthracis (4889)

S. aureus: Overnight culture
B. species: 72 hrs culture
(sporulating media)

•NA Extraction Kits

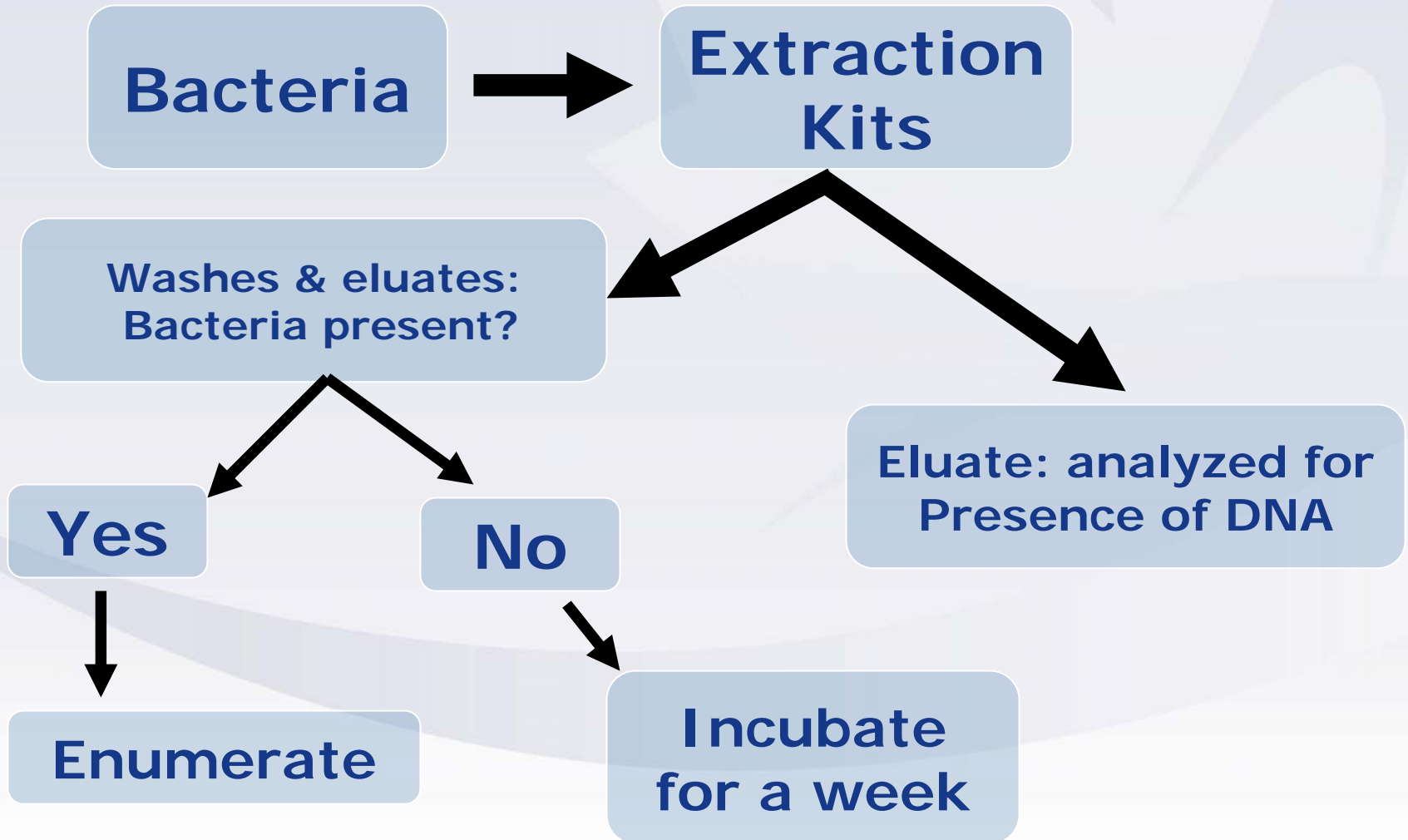
DNeasy Blood & Tissue

DNeasy Plant

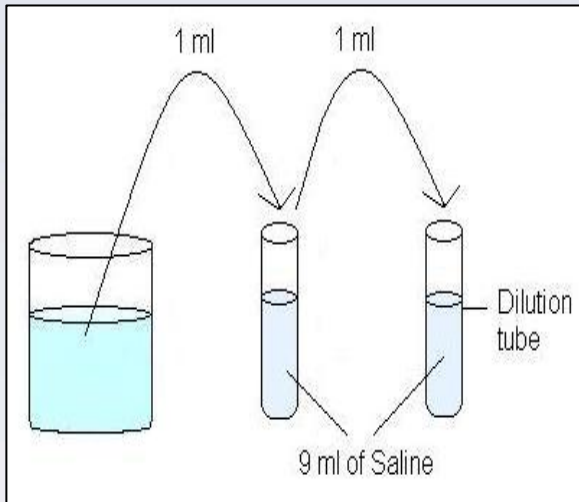
DNAzol

Genomic Wizard

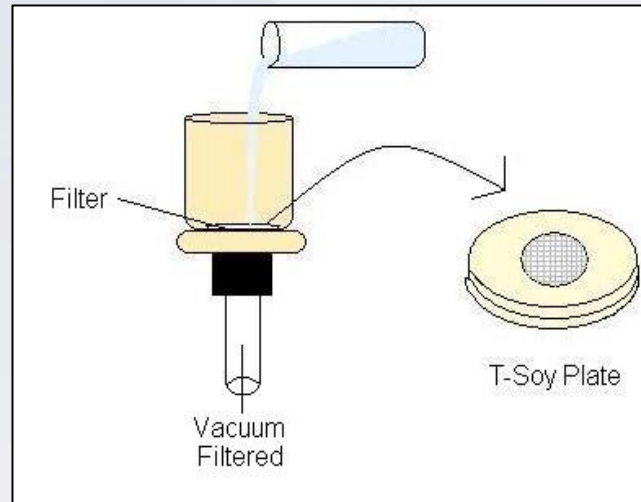
Materials and Methods



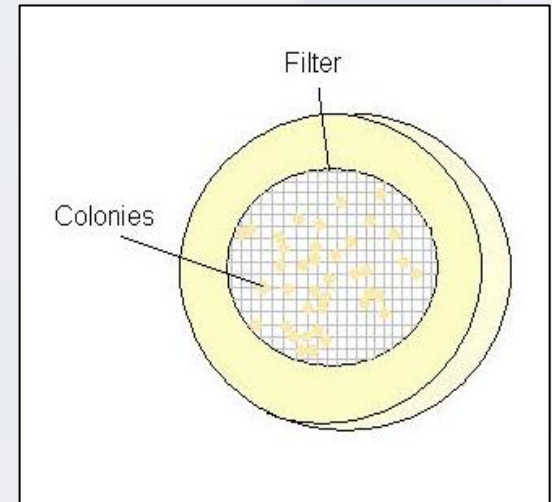
Enumeration of viable bacteria



Serial ten fold dilutions



Filter the dilutions and plate the filters for bacterial growth



Count bacterial colonies

DNeasy Mini Procedure

Sample



Lyse



Bind



Wash



Elute



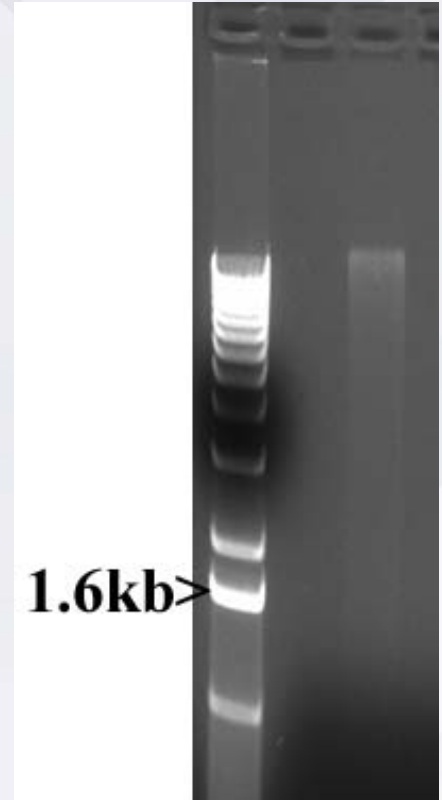
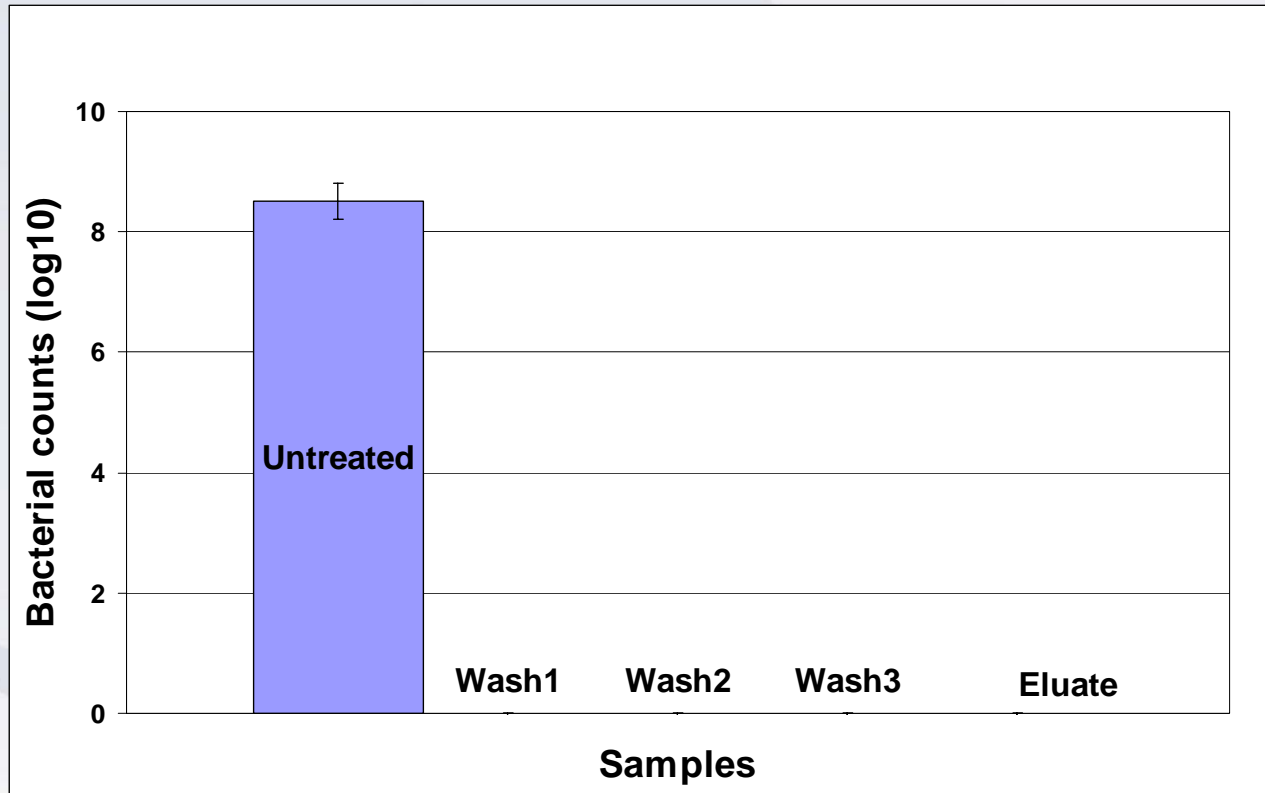
Ready-to-use DNA

DNeasy Blood and Tissue (Qiagen)

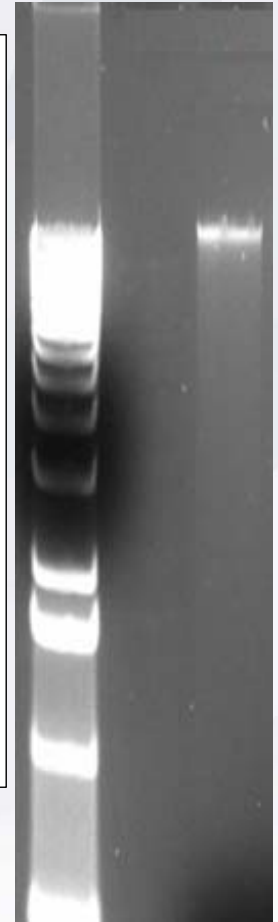
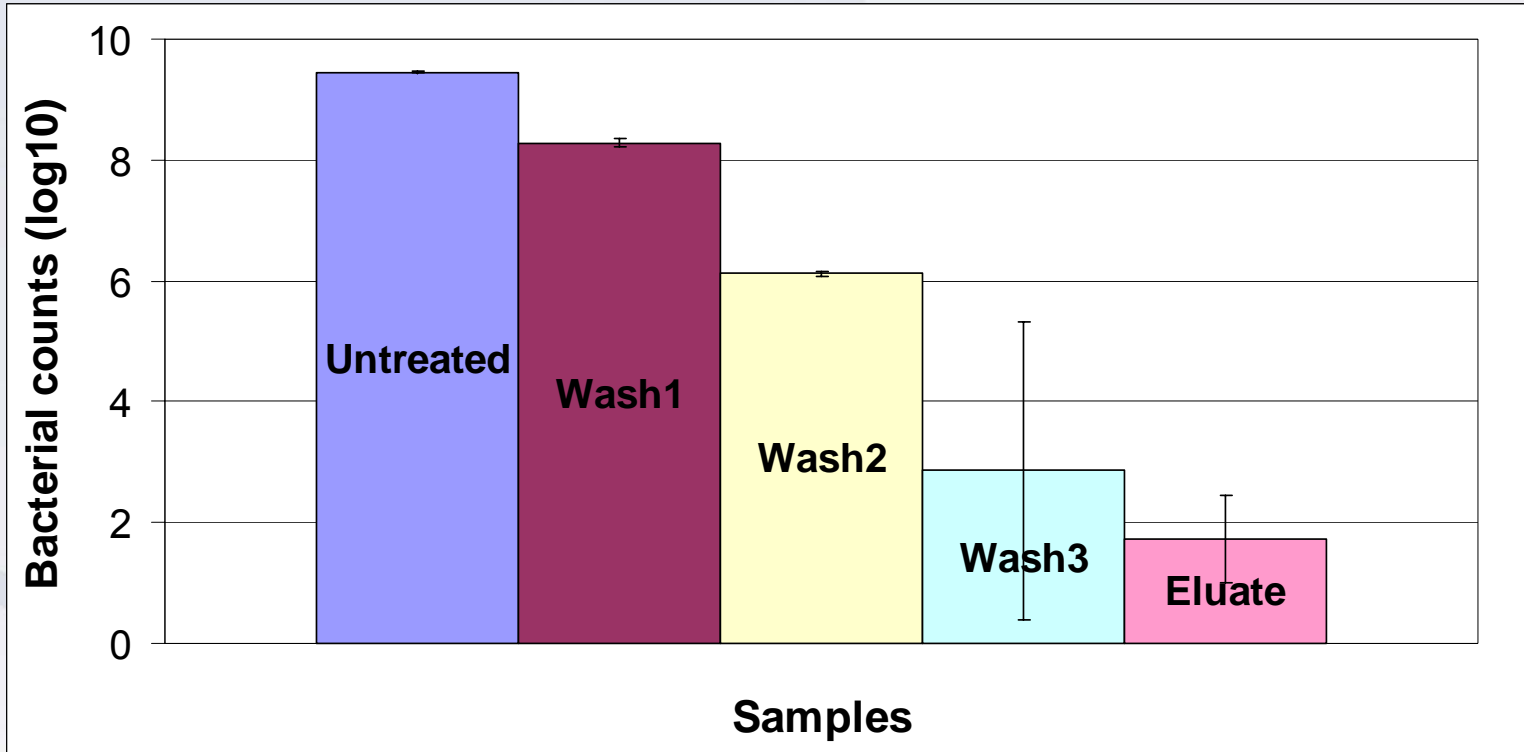
DNeasy combines the properties of a silica based membrane with simple micro spin technology

- lyse cells with enzymatic lysis buffer (**optional microbial inactivation of step @ 95°C for 15 min**)
- DNA is selectively bound to the Dneasy membrane as contaminants pass through (**Discard 1**)
- DNA is washed (**Discards 2 and 3**) to remove remaining contaminants
- DNA is eluted (**Eluate**)

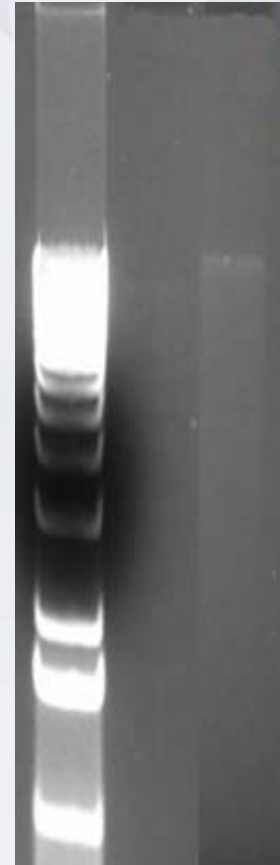
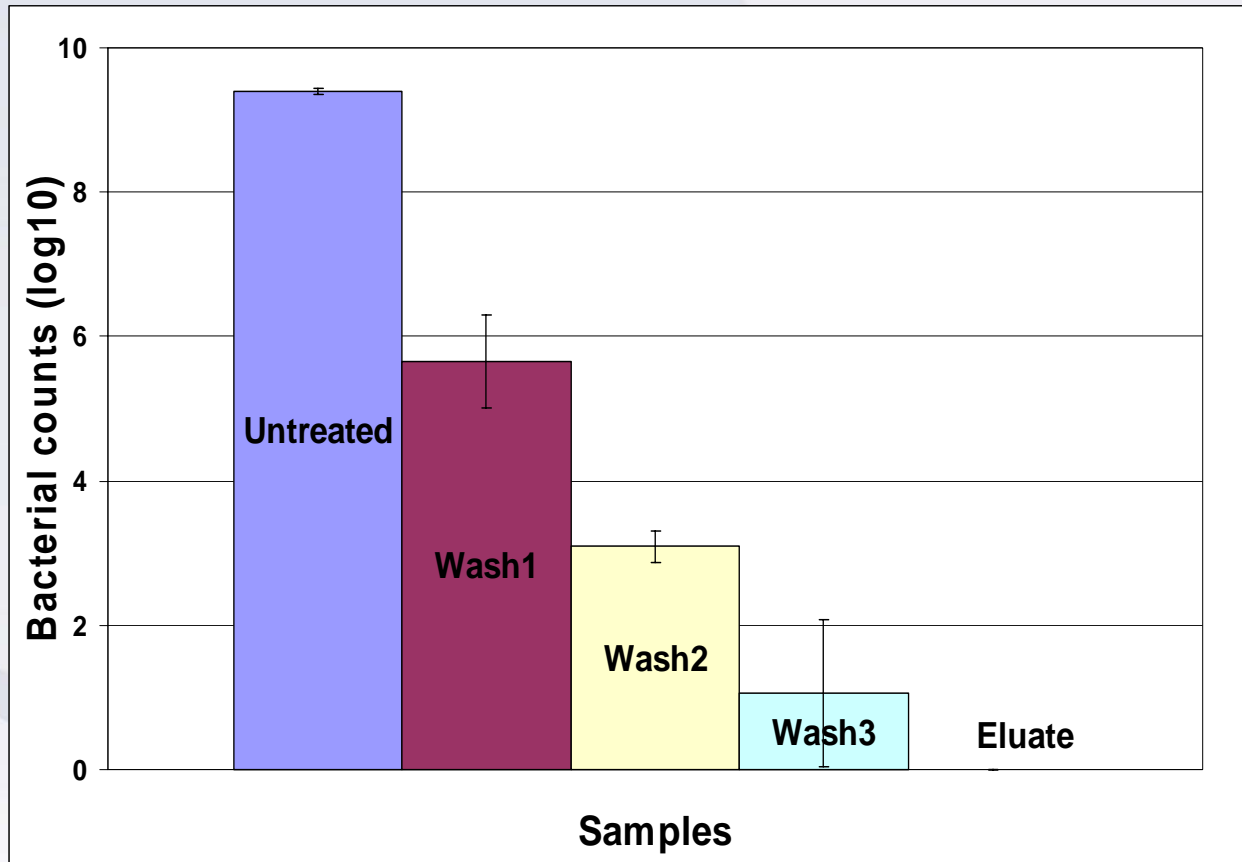
DNeasy: *S. aureus*



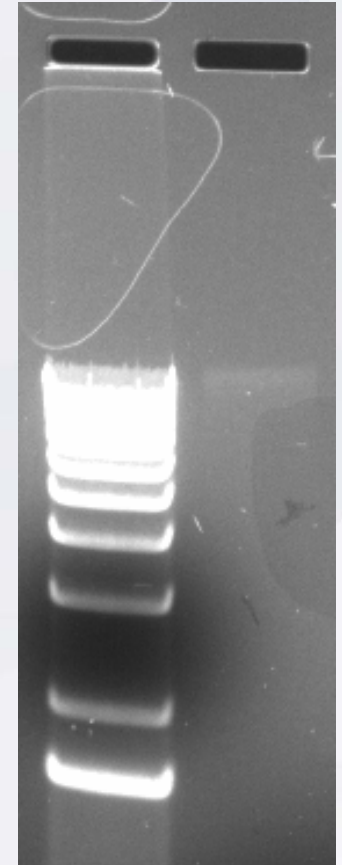
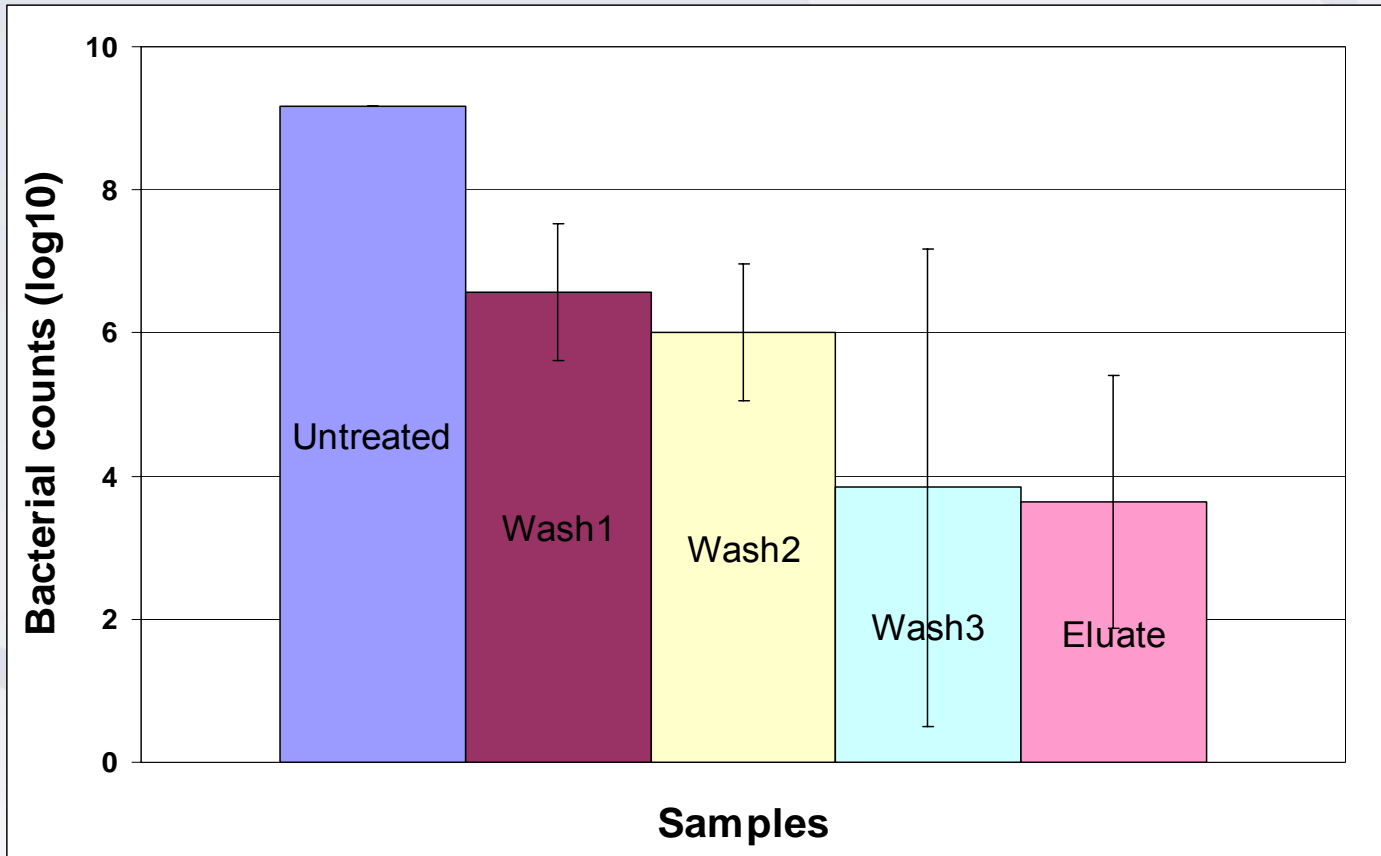
DNeasy: *B. atrophaeus*



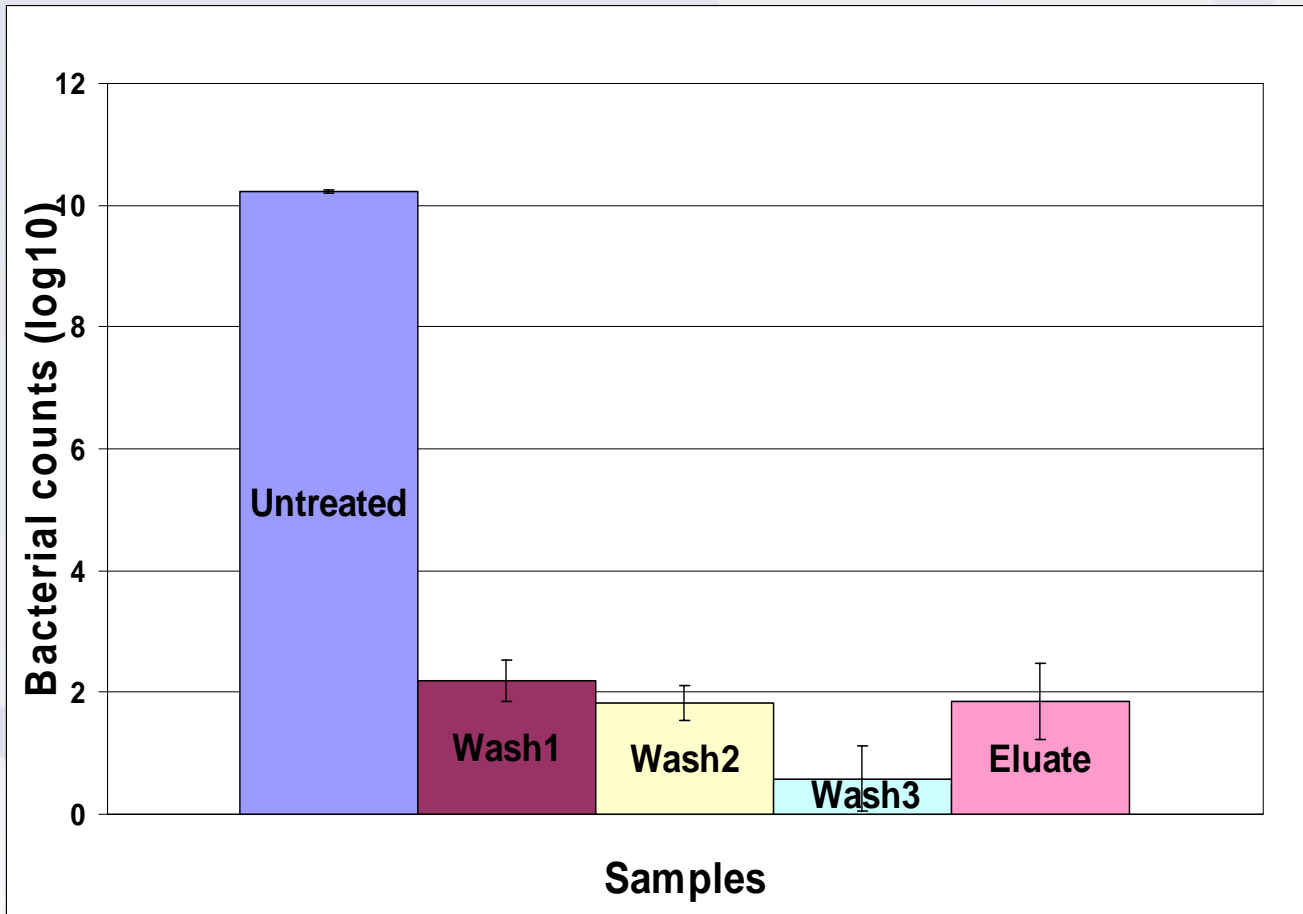
DNeasy: *B. atrophaeus* with Heat Inactivation (95°C for 15')



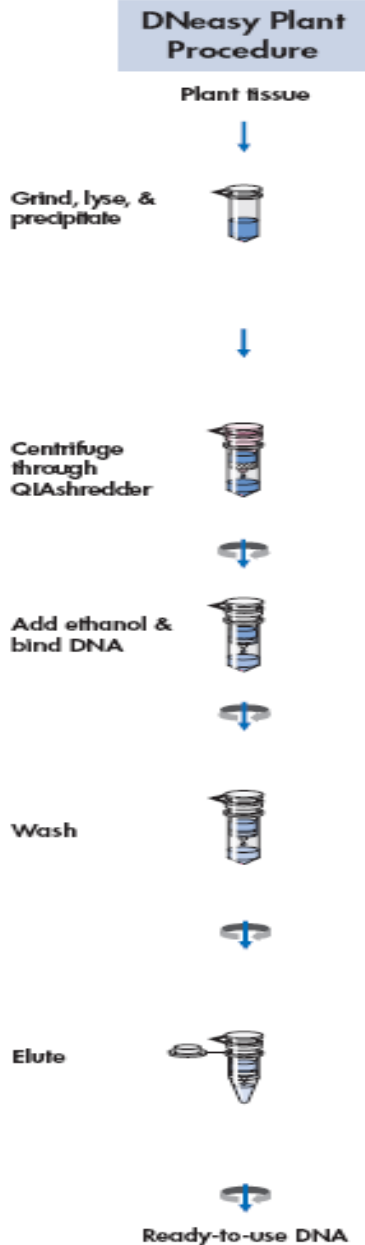
DNeasy: *B. anthracis* No Heat Inactivation



DNeasy: *B. anthracis* 95°C for 15' Heat Inactivation



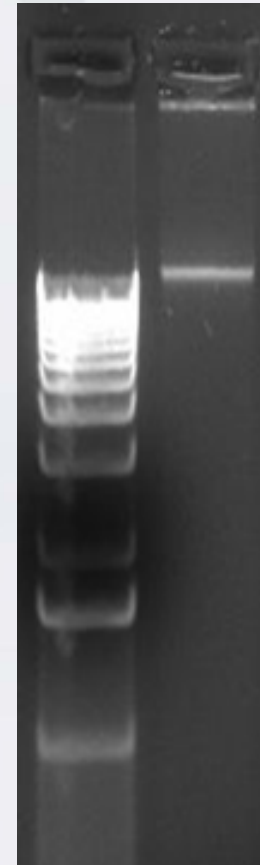
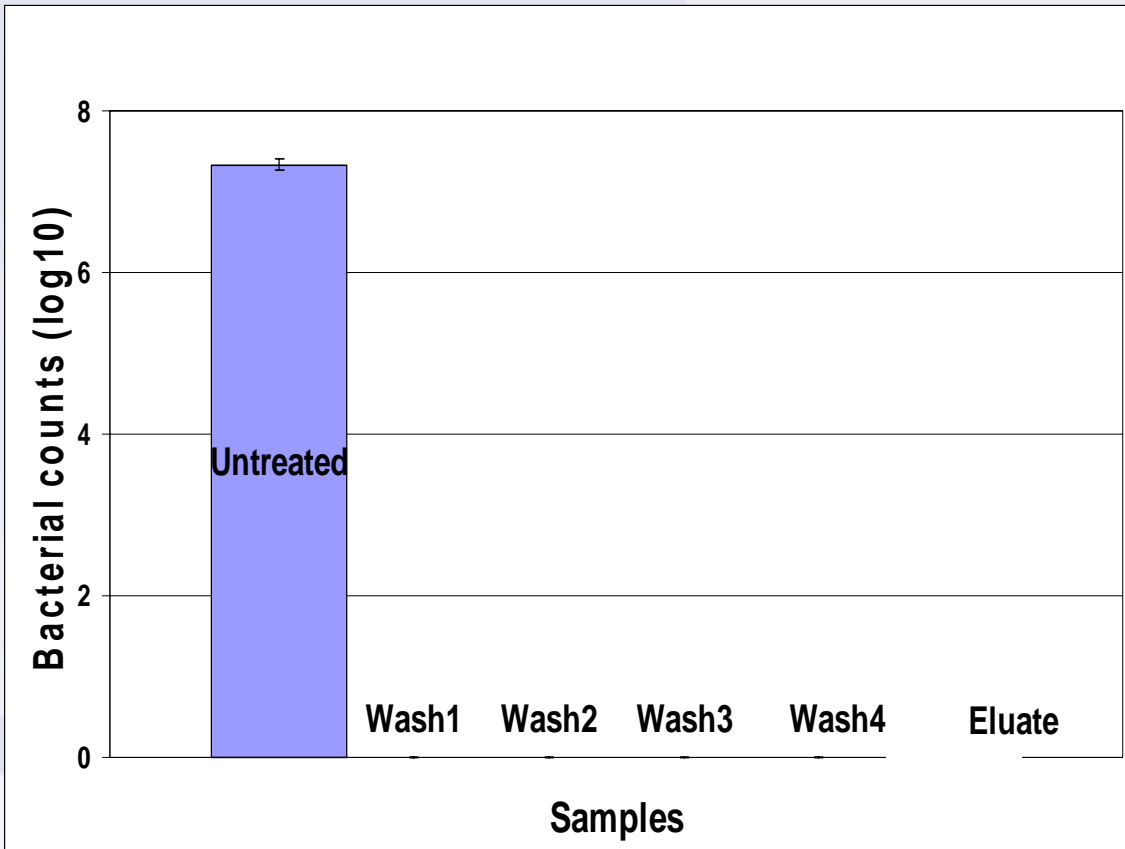
DNeasy Plant (Qiagen)



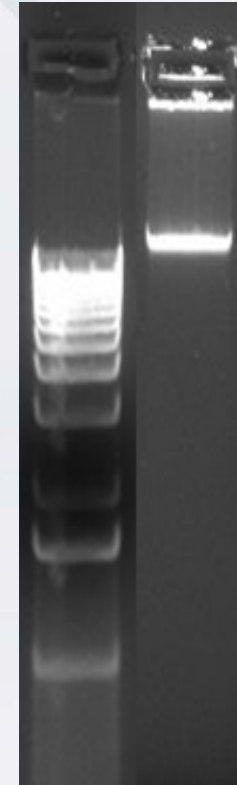
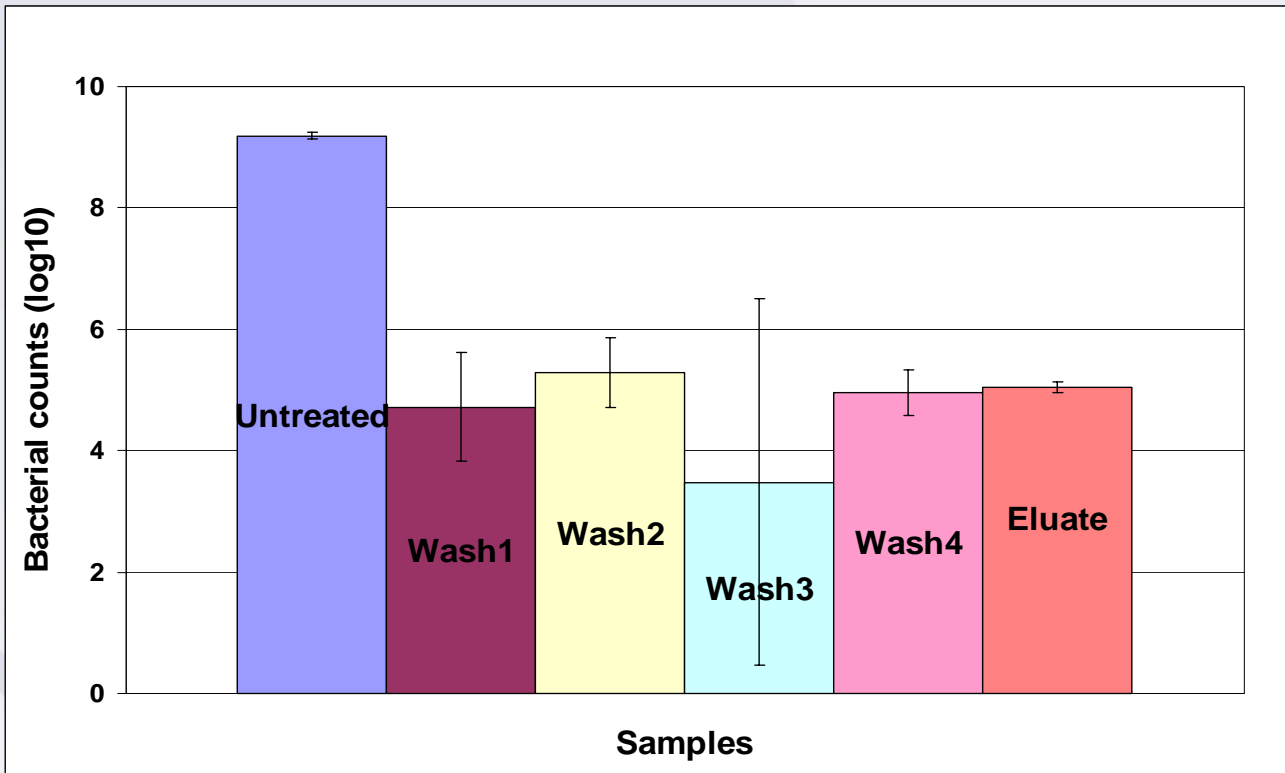
Same as Dneasy Blood and Tissue, but with two separate spin columns and additional washes, to further eliminate remaining contaminants.

- lyse cells and Precipitate detergent, protein, and polysaccharides (**Discard 1**)
- DNA is selectively bound to the Dneasy membrane as contaminants pass through by a series ethanol based washes (**Discards 2, 3, 4**)
- DNA is eluted (**Eluate**)

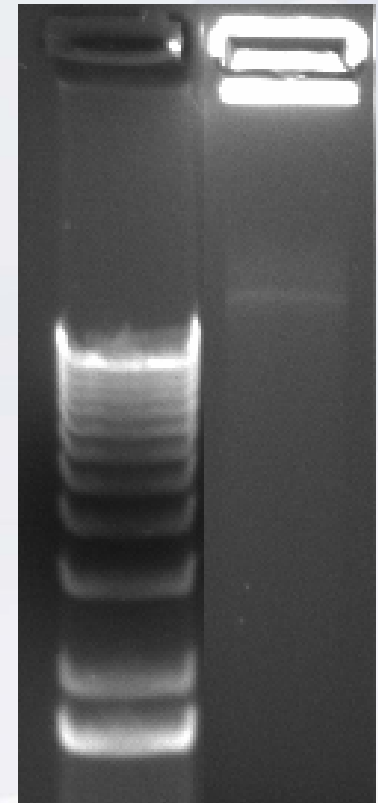
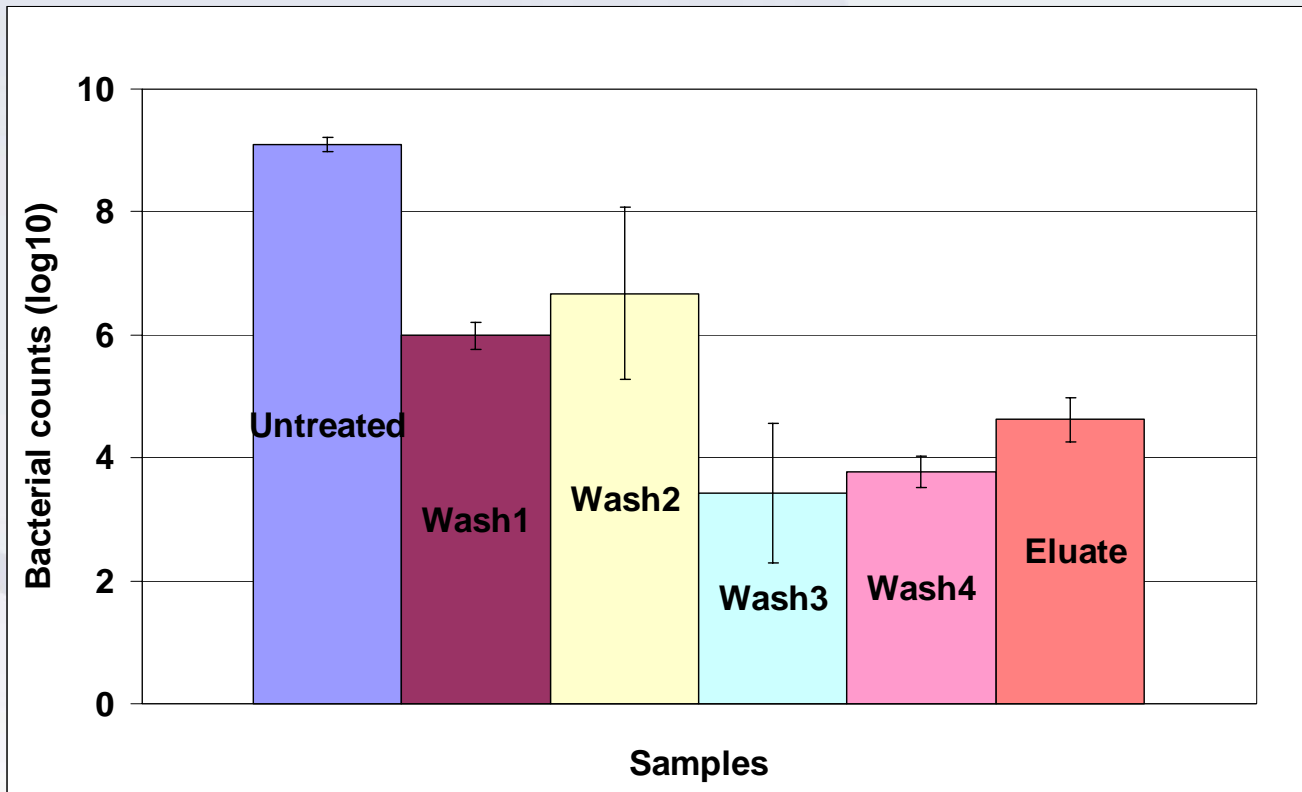
DNeasy Plant: *S. aureus*



DNeasy Plant: *B. atrophaeus*



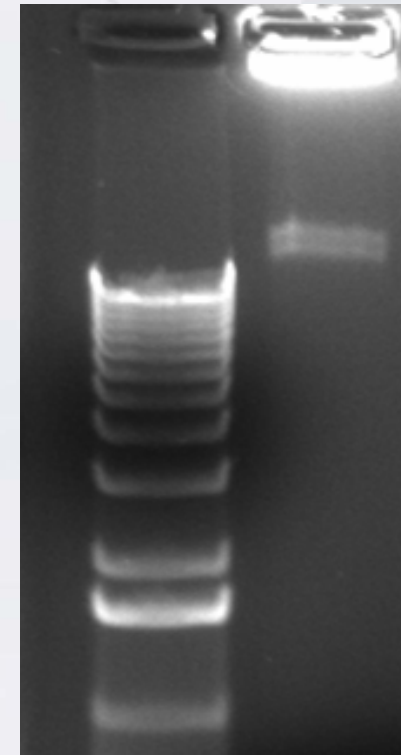
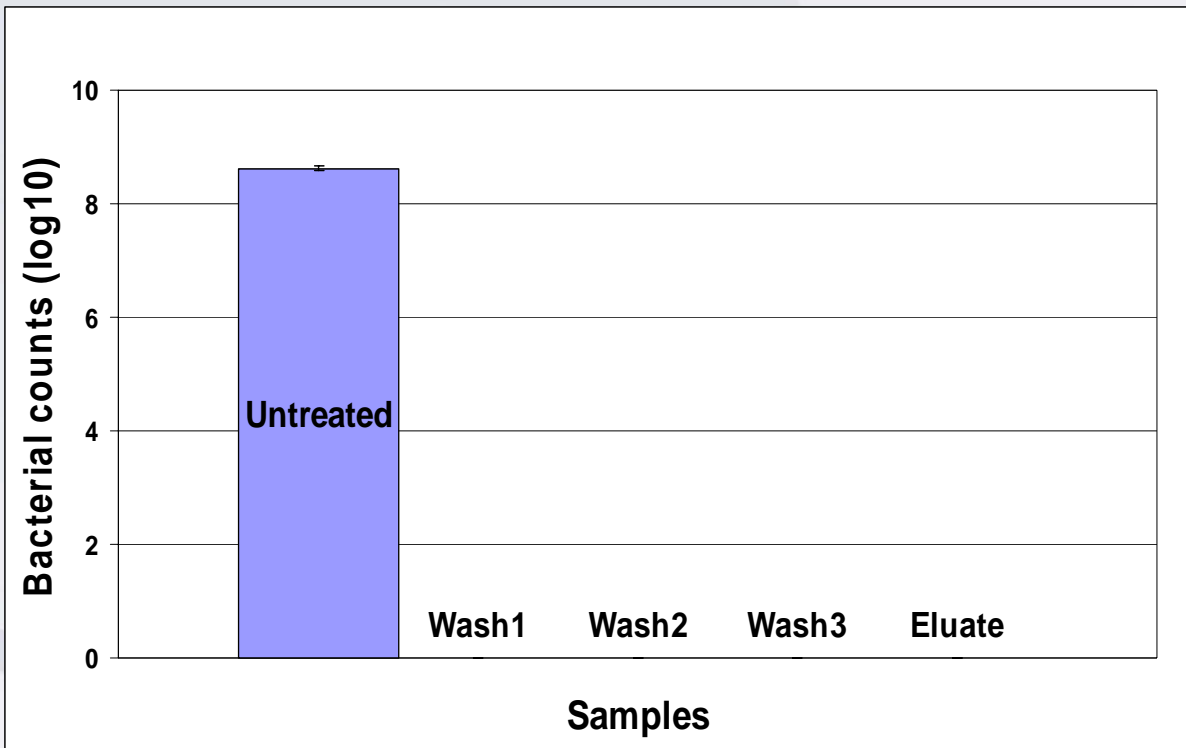
DNeasy Plant: *B. anthracis*



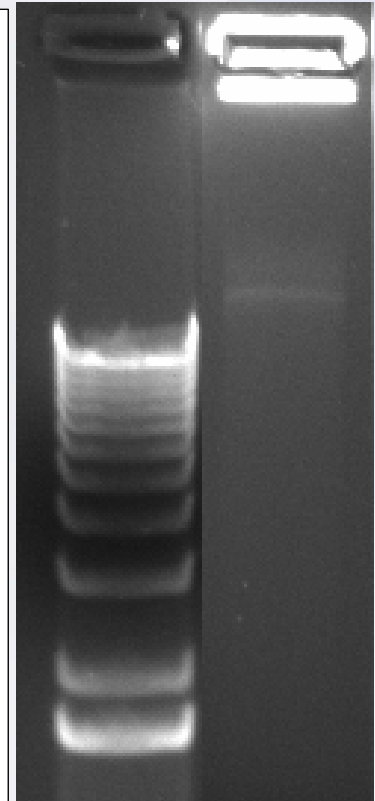
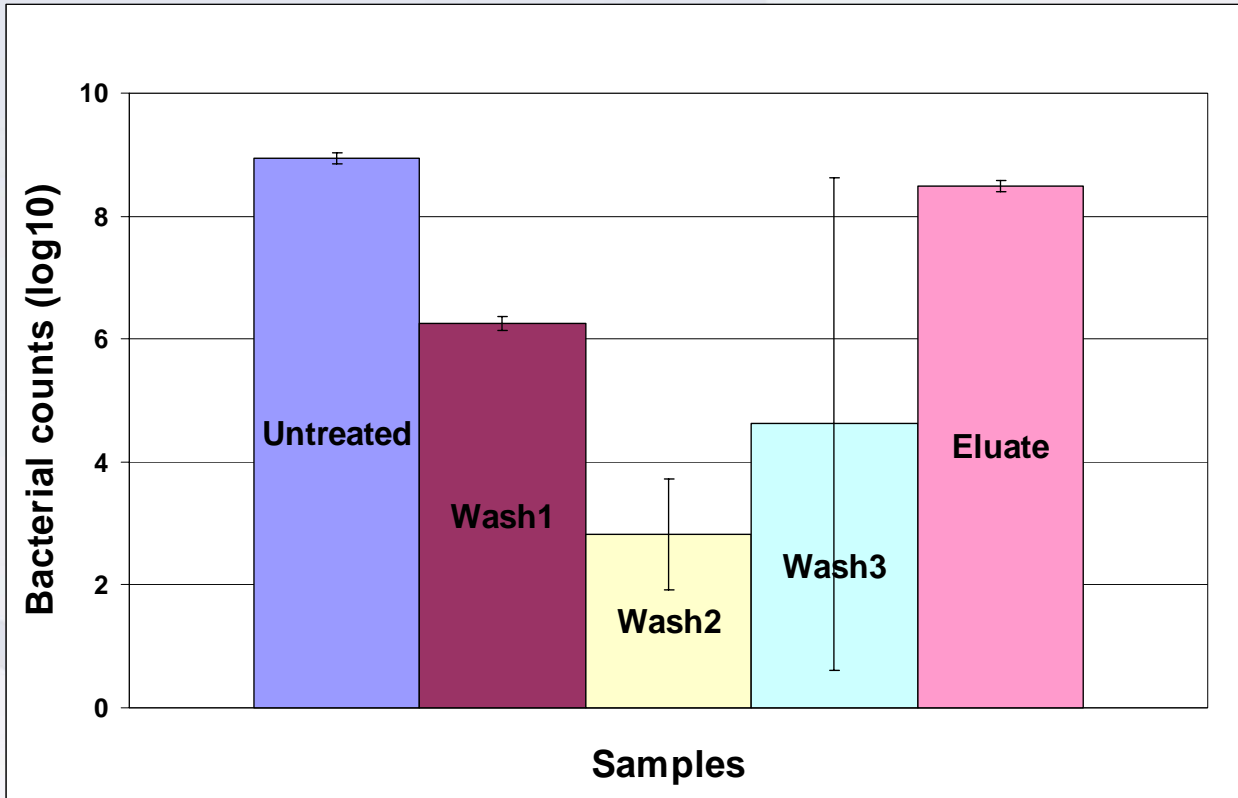
DNAzol (Invitrogen)

- DNAzol reagent procedure is based on a guanidine-detergent lysing solution which permits selective precipitation of DNA from a cell lysate
- DNAzol reagent lyses cells and the remaining cell contaminants are discarded (**Discard 1**)
- DNA is precipitate out of the cell lysate and is cleared of cell lysate by washes (**Discards 2, 3**)
- DNA is then re-dissolved in water or buffer (**Eluate**)

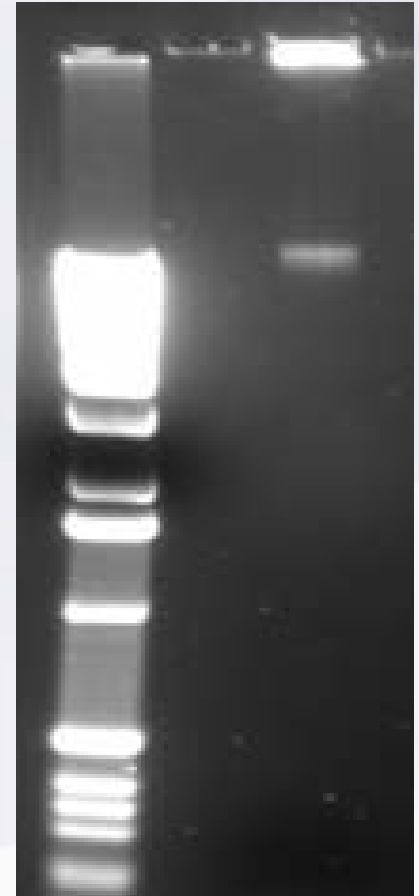
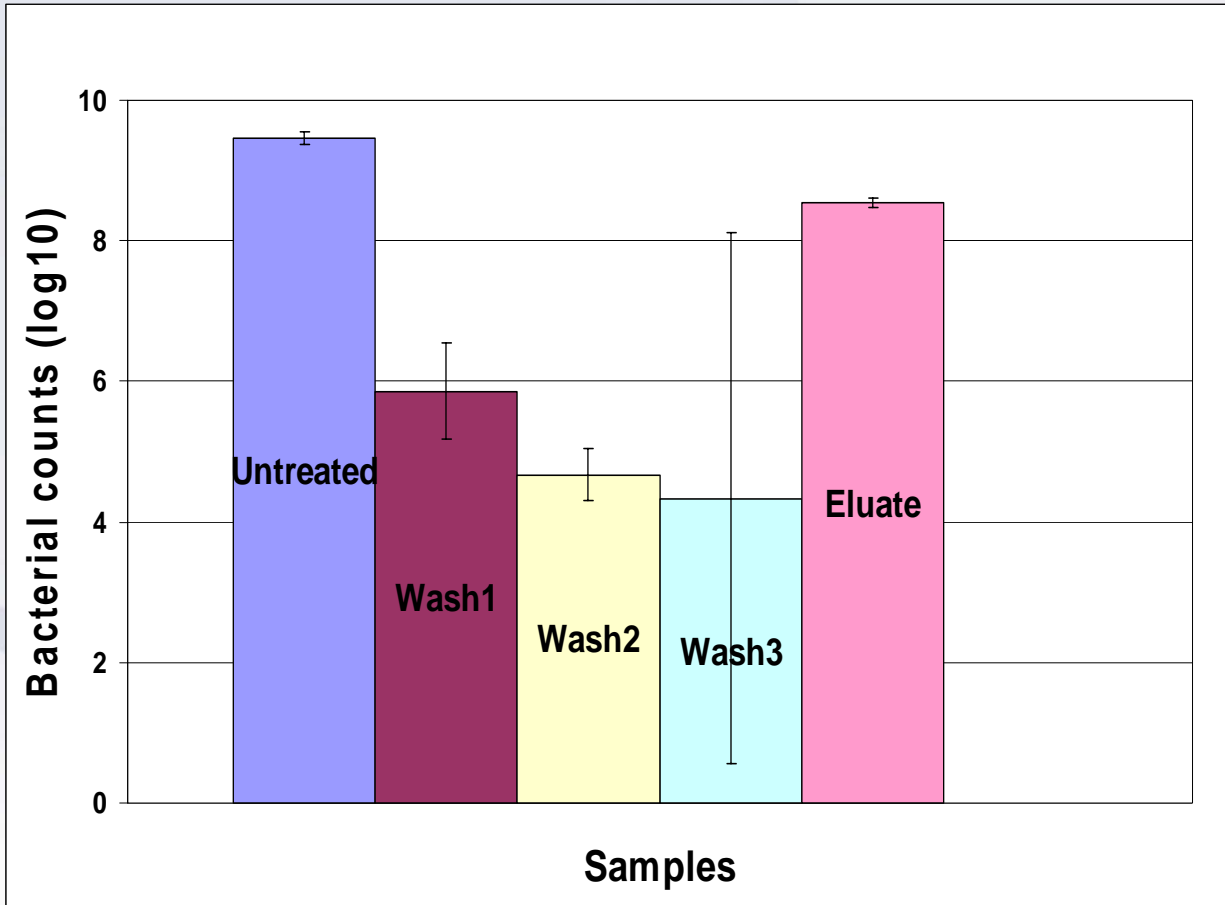
DNAzol: *S. aureus*



DNAzol: *B. atrophaeus*



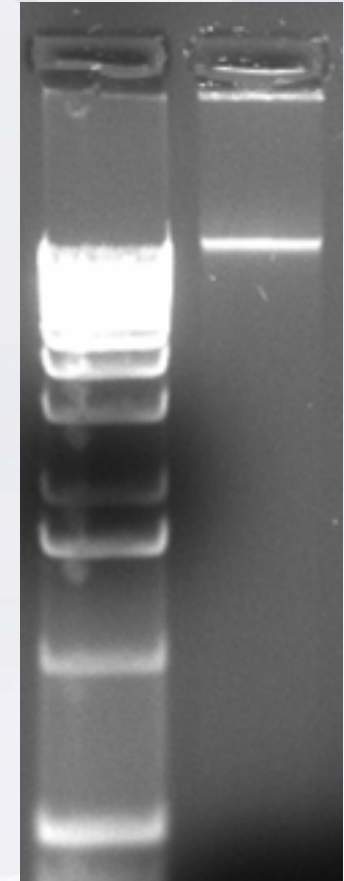
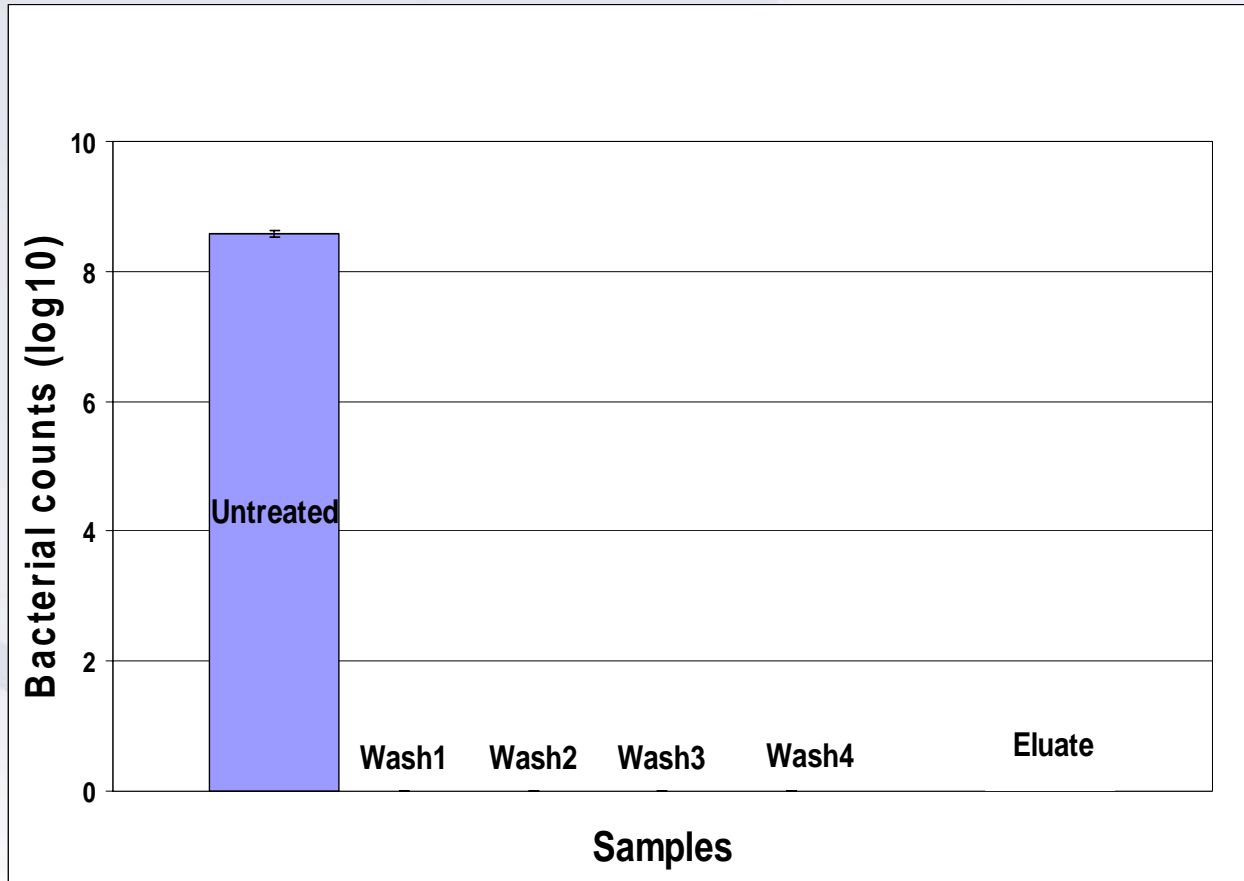
DNAzol: *B. anthracis*



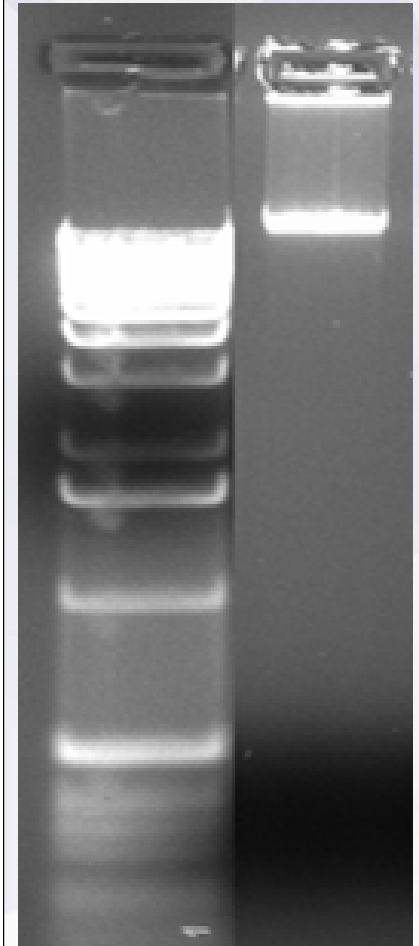
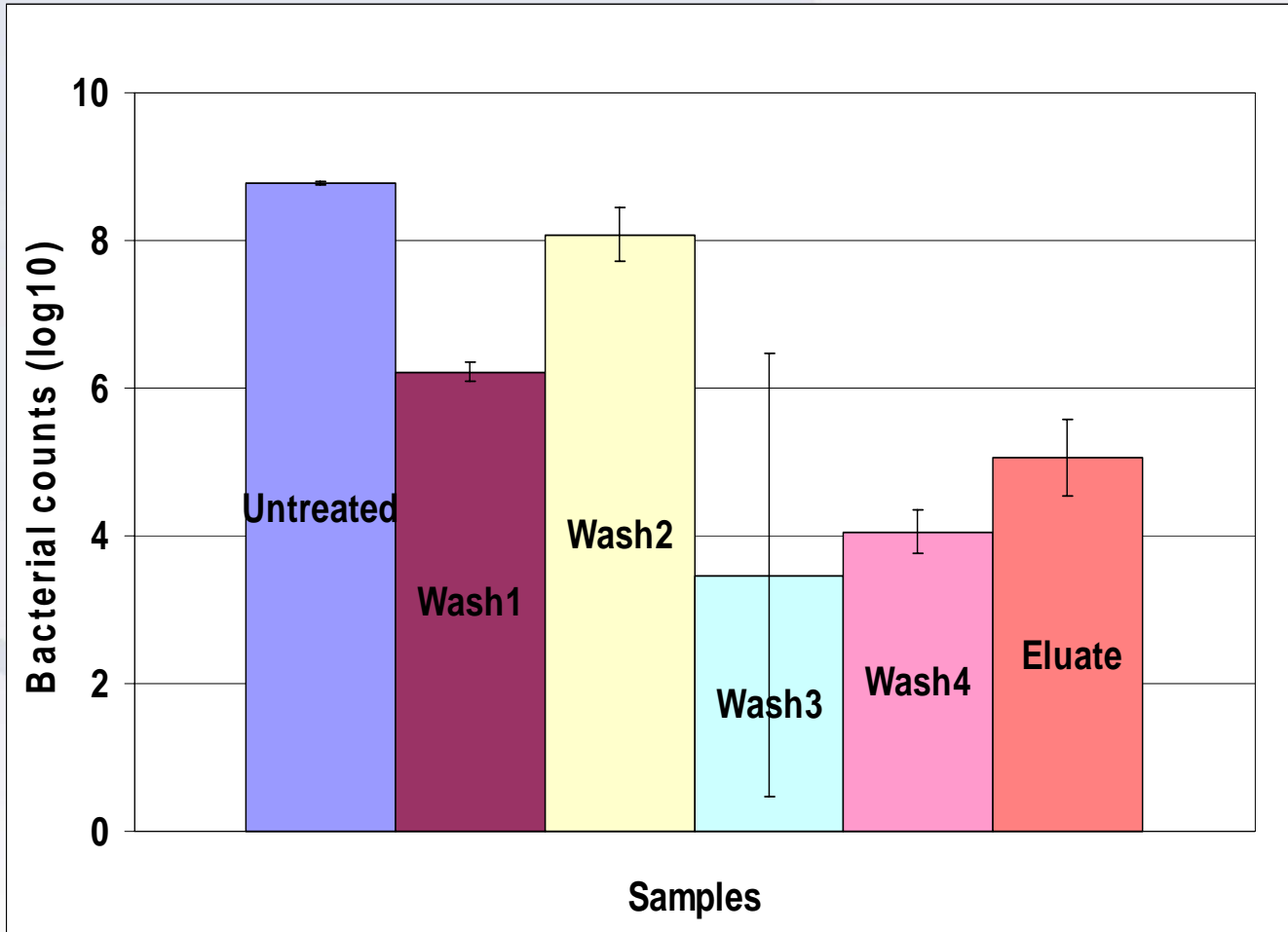
Genomic Wizard (Promega)

- The cells and cell nuclei are lysed and cellular proteins are removed by a salt precipitation that leaves the high molecular weight genomic DNA in solution
- genomic DNA is then desalted by isopropanol precipitation and is washed to remove remaining contaminants (**Discards 1-4**)
- DNA is then re-dissolved in water/buffer (**Eluate**)

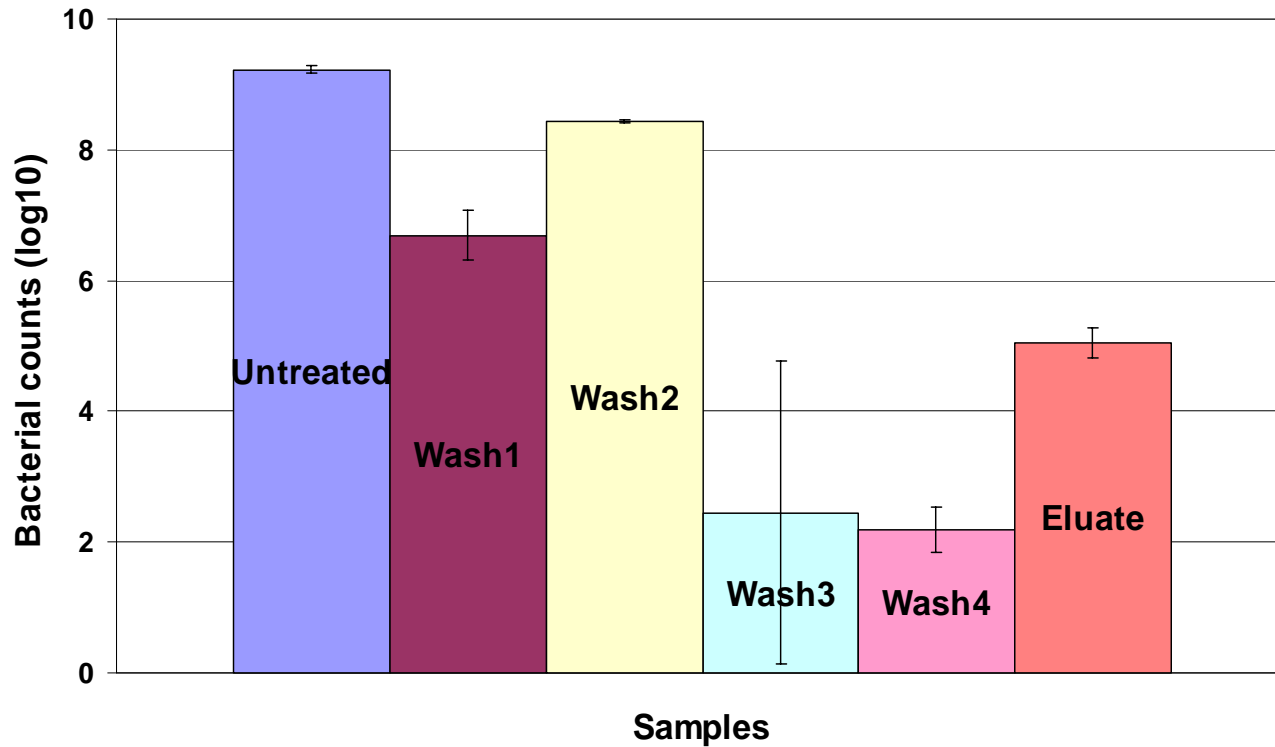
Genomic Wizard: *S. aureus*



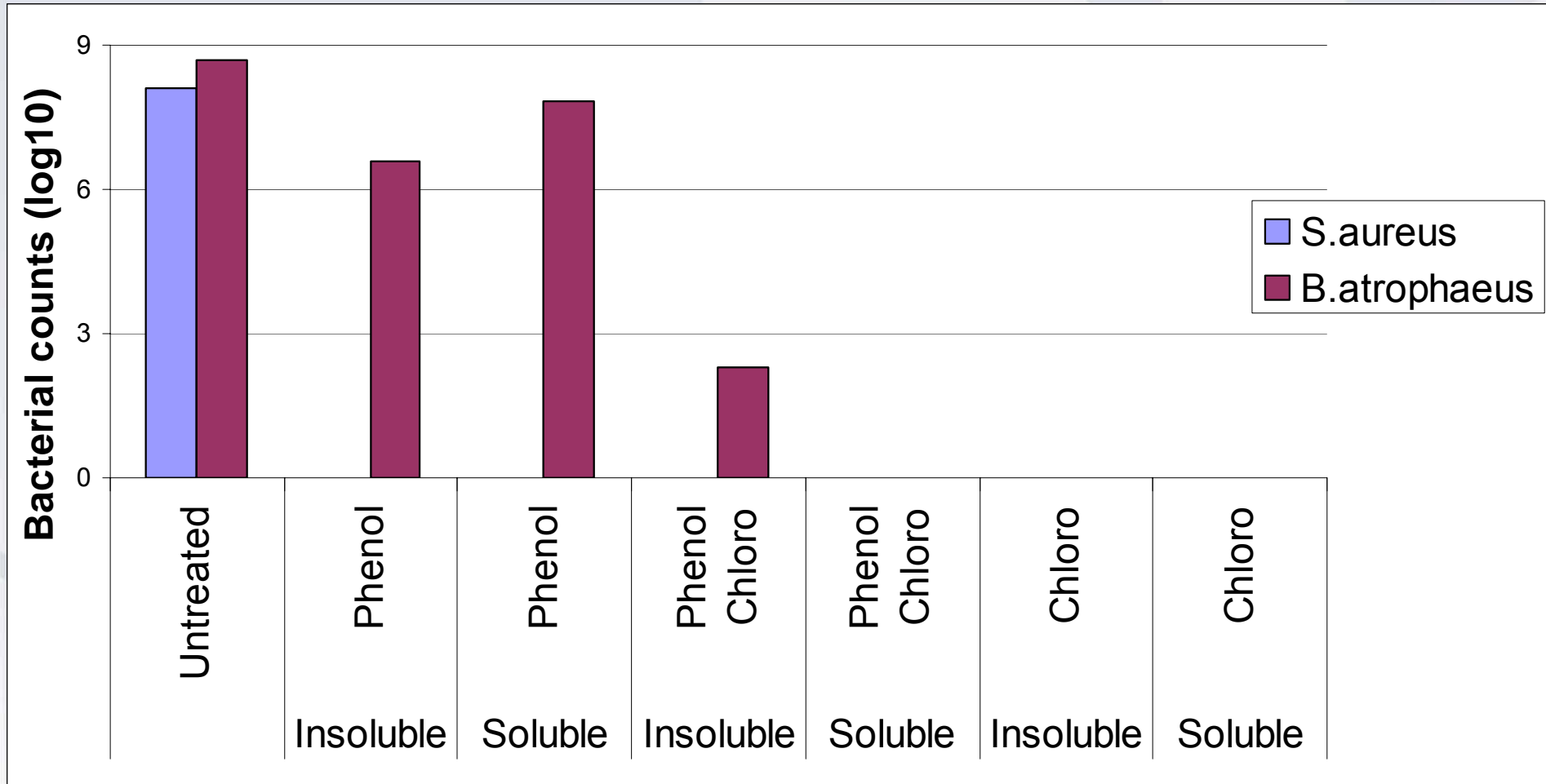
Genomic Wizard: *B. atrophaeus*



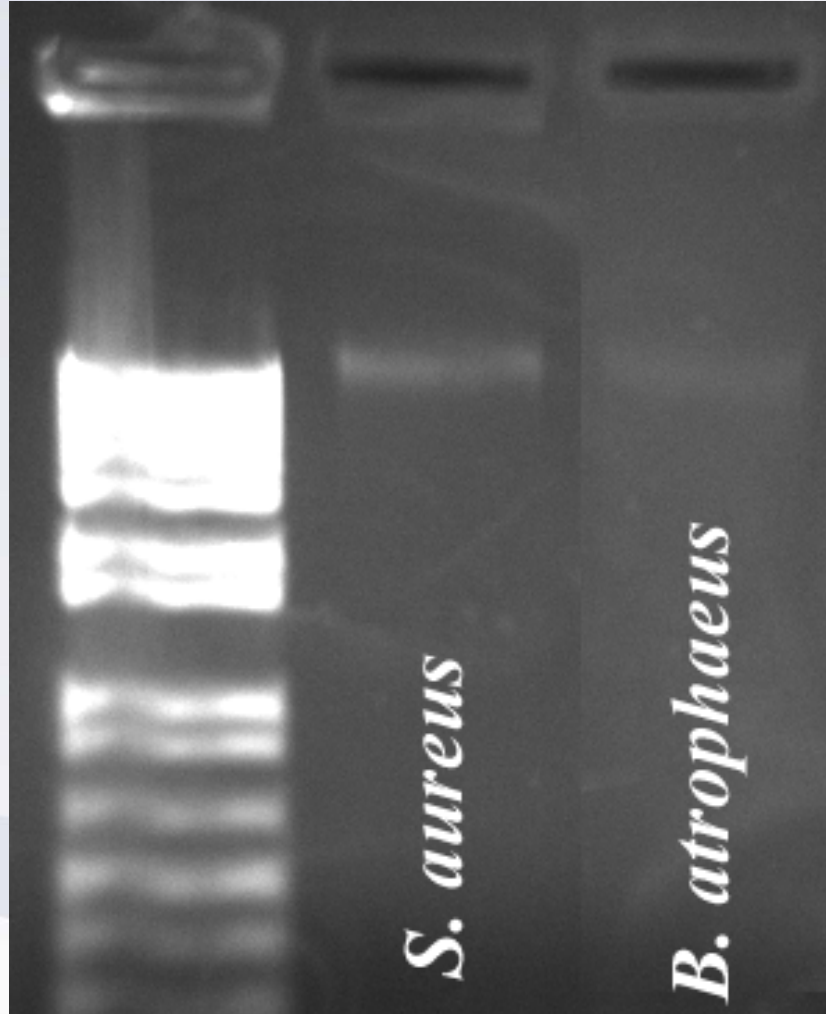
Genomic Wizard: *B. anthracis*



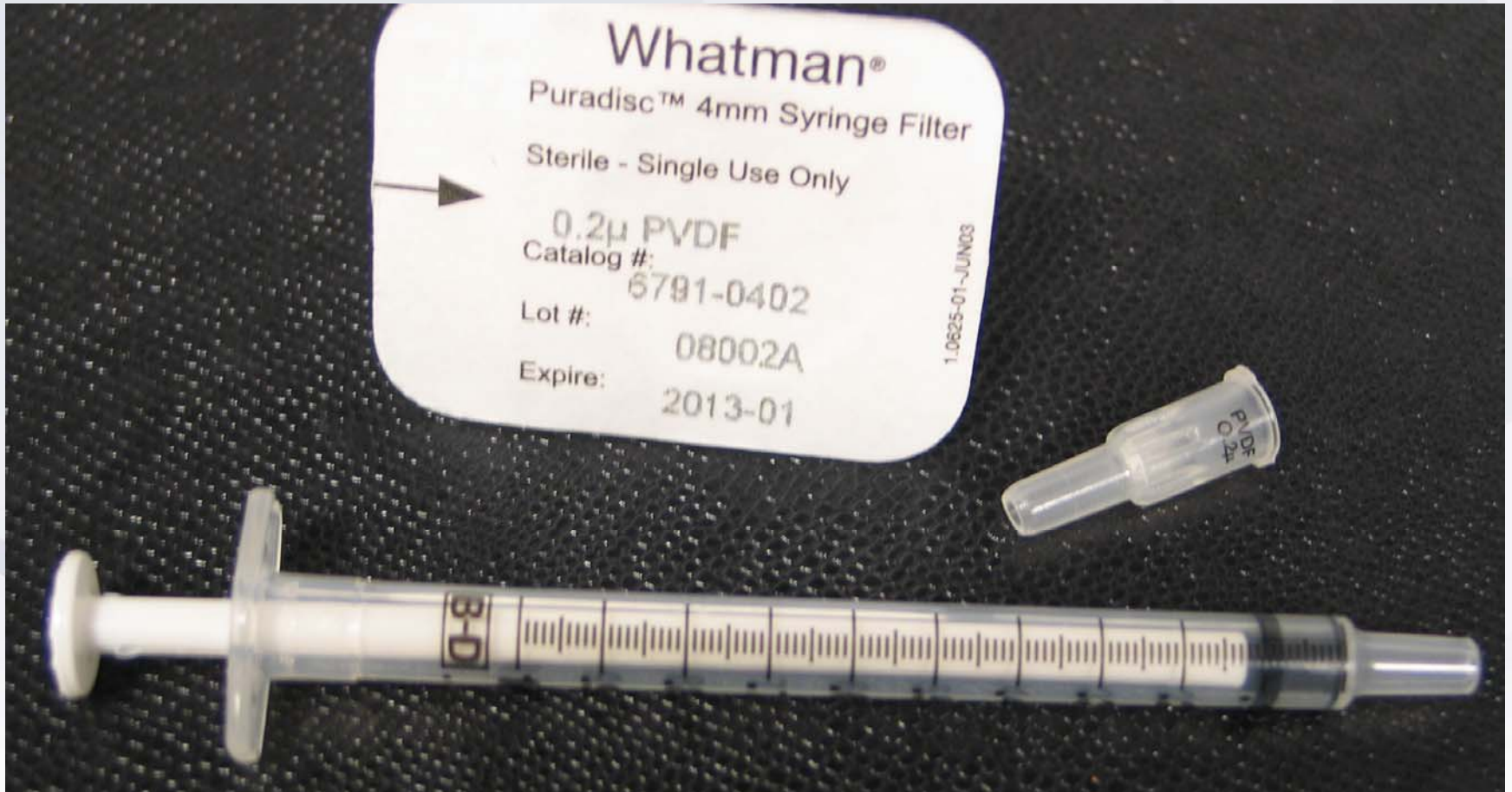
Phenol Chloroform Extraction



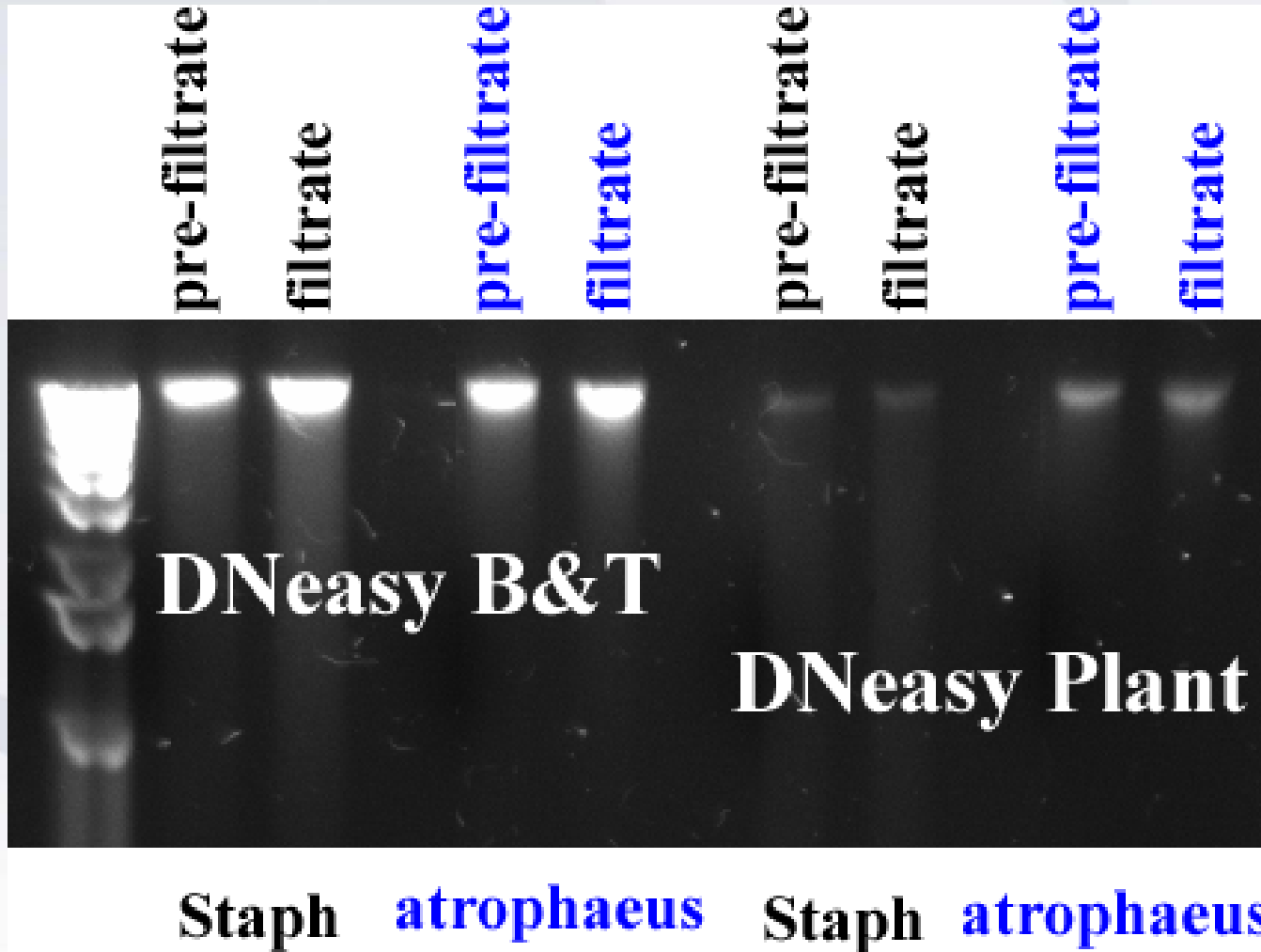
Phenol Chloroform Extraction



Bacteriological Filter to Remove Bacteria



Filtration Results



Conclusions

- All four kits and phenol chloroform method successfully extracted DNA from the three bacterial species studied
- Non-spore forming bacterium, *S. aureus* was inactivated by all the extraction methods
- However, the spore forming bacteria *B. atrophaeus* and *B. anthracis* were inactivated only by Phenol Chloroform