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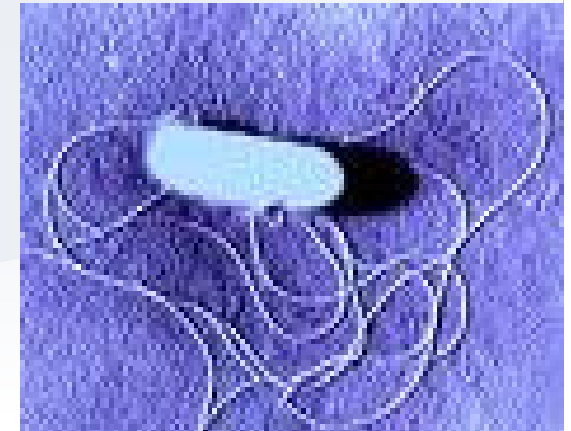
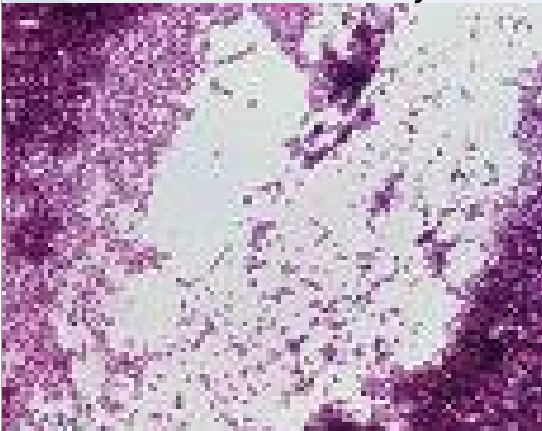
Comparative Inactivation Studies of *Listeria Monocytogenes* Using Commercially Available Disinfectants at Room and Refrigeration Temperatures

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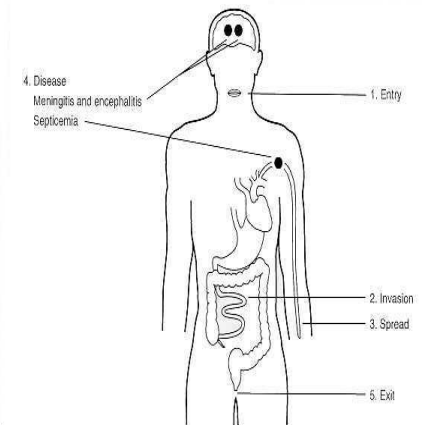
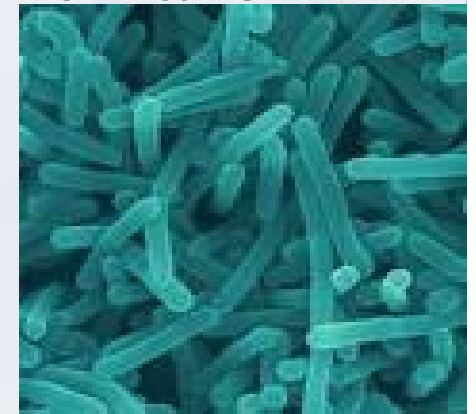
Listeria monocytogenes

- Short, motile, facultative anaerobic, nonsporulating, gram positive bacillus
- 0.5 to 2 microns x 0.5 microns
- The only human and animal pathogen of the 7 species of *Listeria*
- Ubiquitous in nature and can survive in acidic, cool (4⁰C) and salty environments



Listeriosis

- Listeriosis is an infrequent but serious illness
 - affects the central nervous system of humans and domestic animals
 - Mortality 20-30%
- Acquisition from consumption of contaminated foods
- Incubation period 2-70 days



Listeriosis

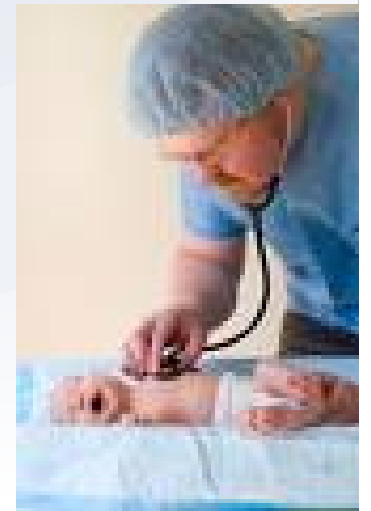
- Symptoms range from:
 - asymptomatic carriers
 - septicemia, meningitis
 - abortion/stillbirth
 - death
- Increase risk groups:
 - Pregnant women
 - Newborns
 - Immunosuppressed
 - Elderly
- Early diagnosis is critical – treatment with Ampicillin or Amoxicillin (3 weeks)



Listeriosis



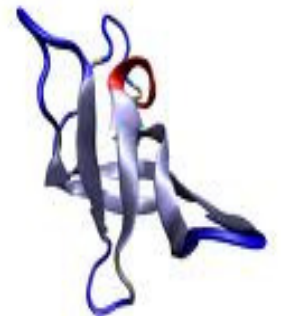
- 11 *L. monocytogenes* Serotypes but 3 cause most infections
 - 4b, 1/2b, and 1/2a cause over 90% of human and animal cases
- Virulence strongly correlated with the production of P60 and listeriolysin O
- Ability to escape T-cell response



Cold Shock Proteins (Csp)



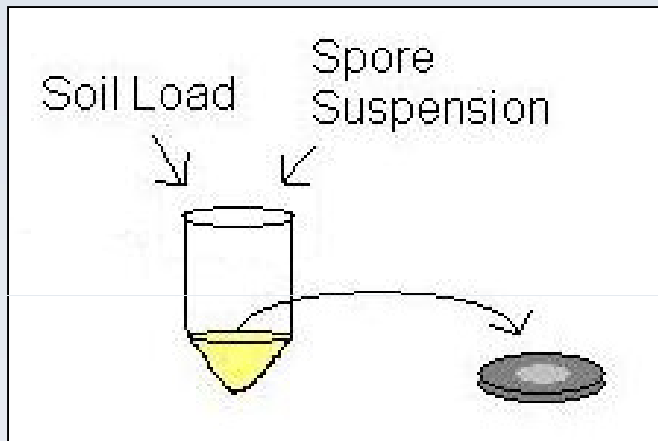
- Functions not well understood
- Most studies with *E.coli* and *B.subtilis*
- *Listeria monocytogenes* has 3 Csp (A,B,D)
- Hierarchy of importance in growth at low temp: CspA>CspD>CspB
- Importance in response to increases in NaCl: CspD>Cspa/CspB



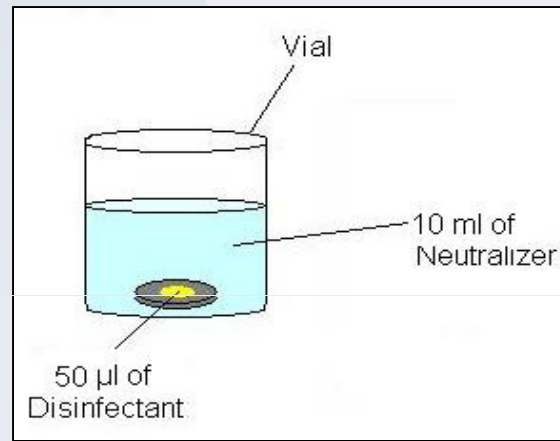
Project Objective

- To determine the bactericidal efficacy of readily available commercial disinfectants and the effect that low temperatures have on their ability to inactivate *Listeria monocytogenes*
 - Ethanol, Percept, Bleach, Microchem and VigorOXusing a standardized testing method
- QCT-2 analysis

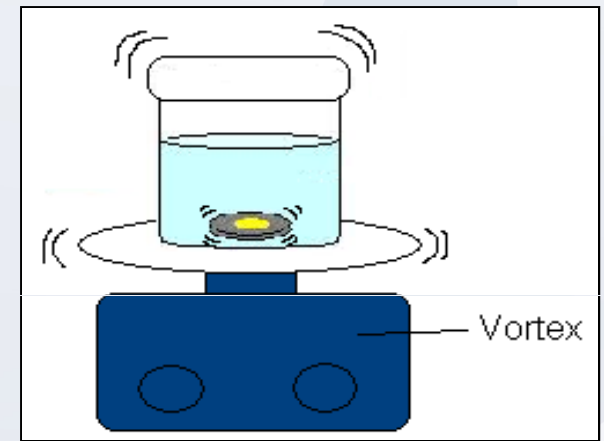
Experimental Methods



Inoculation

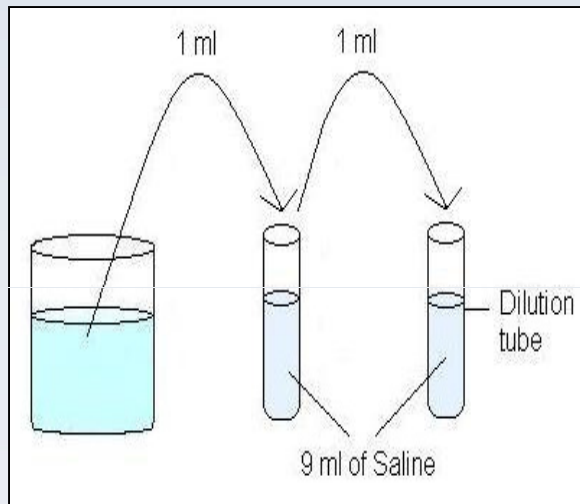


Exposure and
Neutralization

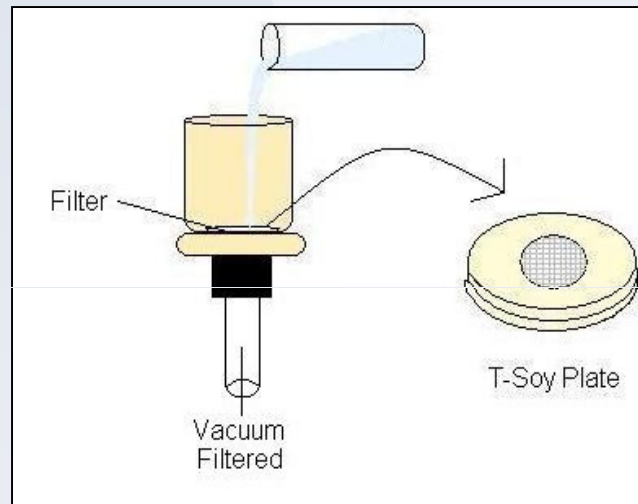


Vortex

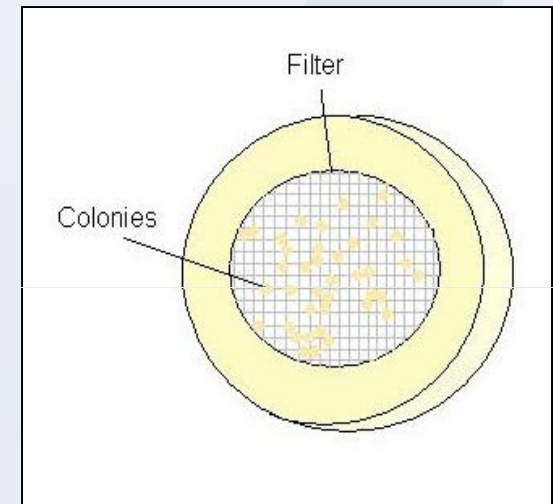
Enumeration of Viable Bacteria



Serial ten fold dilutions



Filter the dilutions and plate the filters for bacterial growth



Count bacterial colonies

Disinfectants Tested

- Ethanol (Alcohol)
- Percept (Accelerated hydrogen peroxide)
- Bleach (Sodium hypochlorite)
- Microchem (Quaternary ammonium cpd)
- VigorOX (5.1% Peracetic acid and 21.7% H_2O_2)



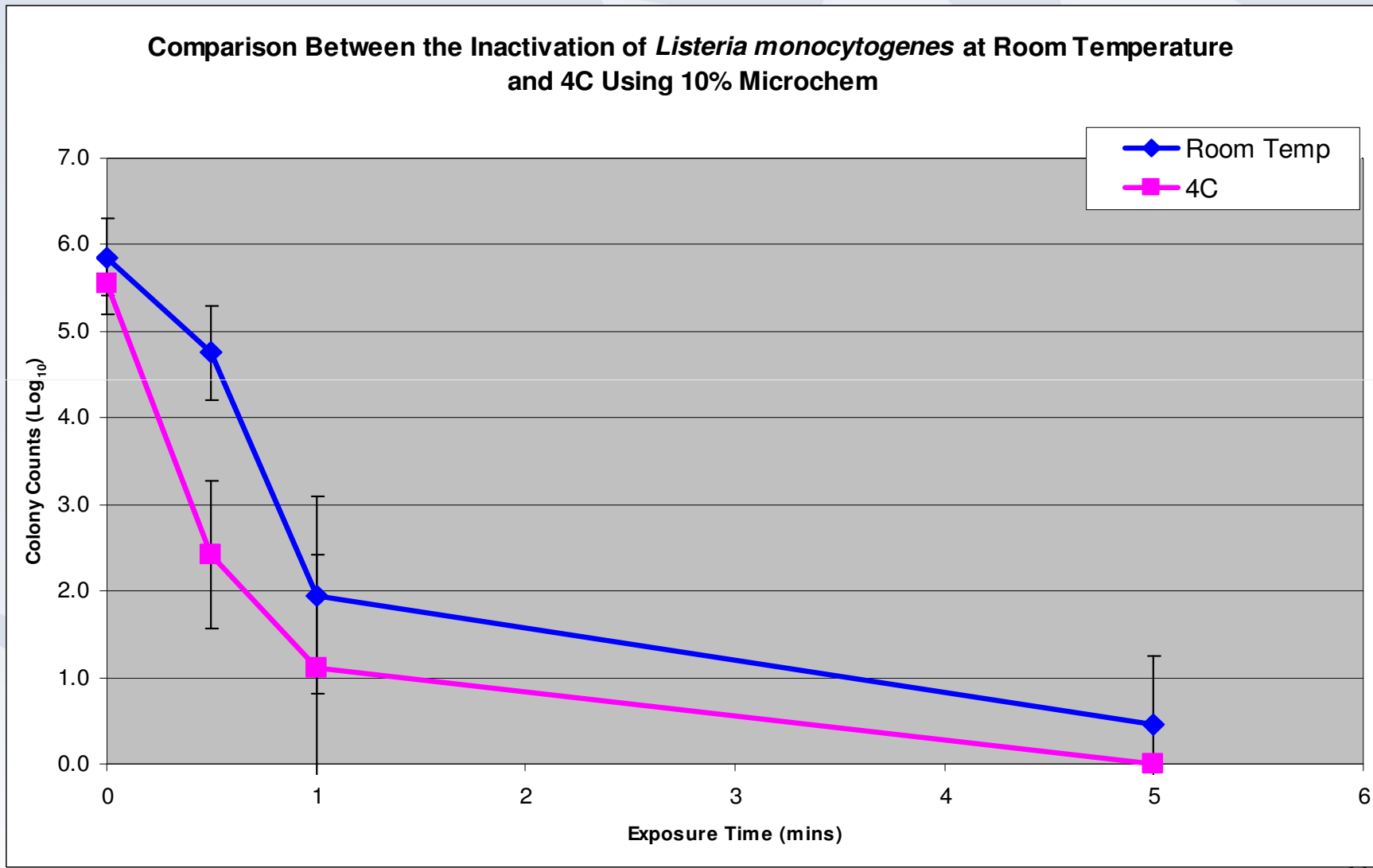
4°C Testing

- The disinfectants and respective concentrations chosen for testing at 4°C were based on the disinfectants performance in previous room temperature experiments. The most relevant disinfectants were used at a concentration that would allow comparisons between RT and 4 °C.

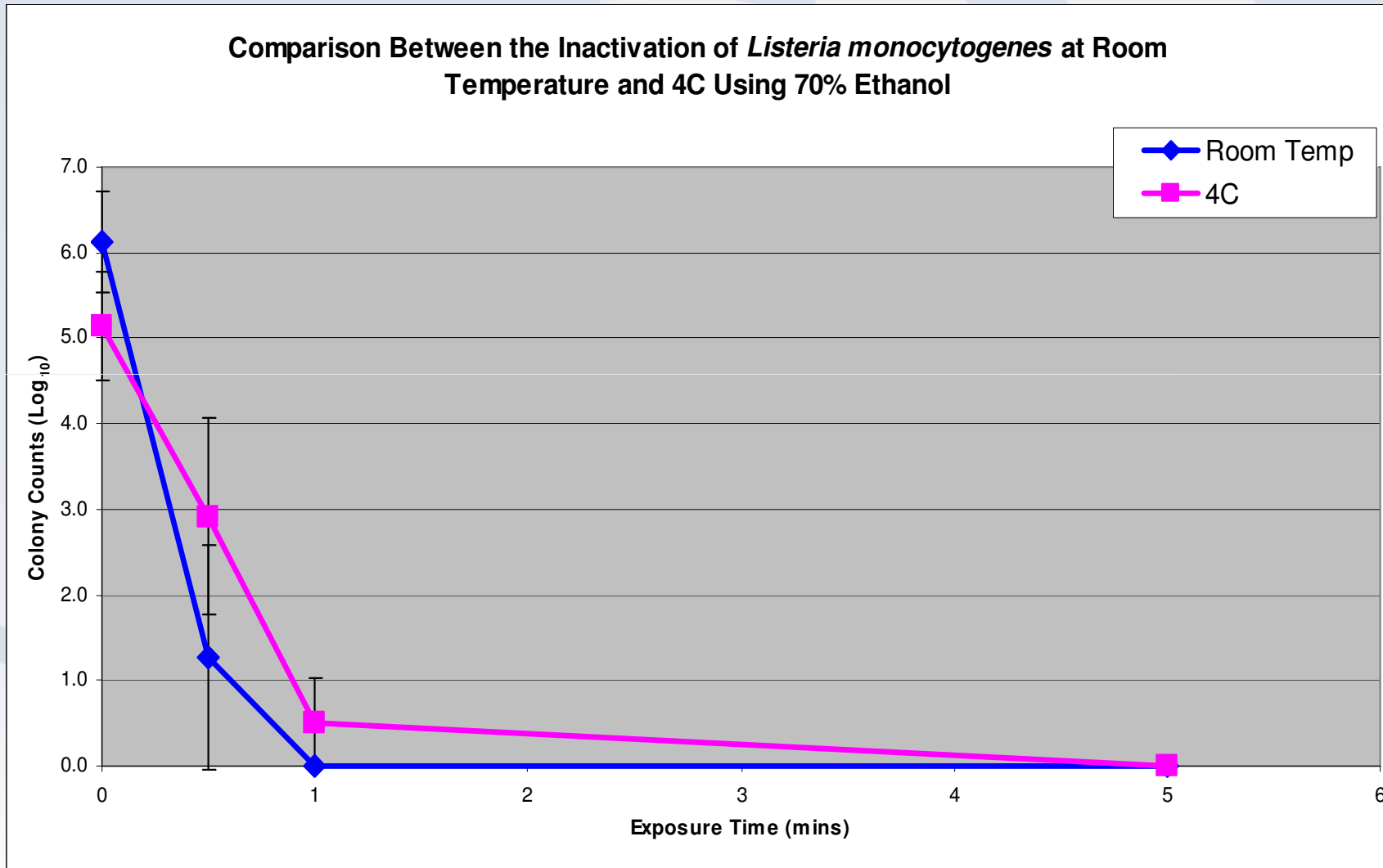


10% Microchem

Comparison Between the Inactivation of *Listeria monocytogenes* at Room Temperature and 4C Using 10% Microchem

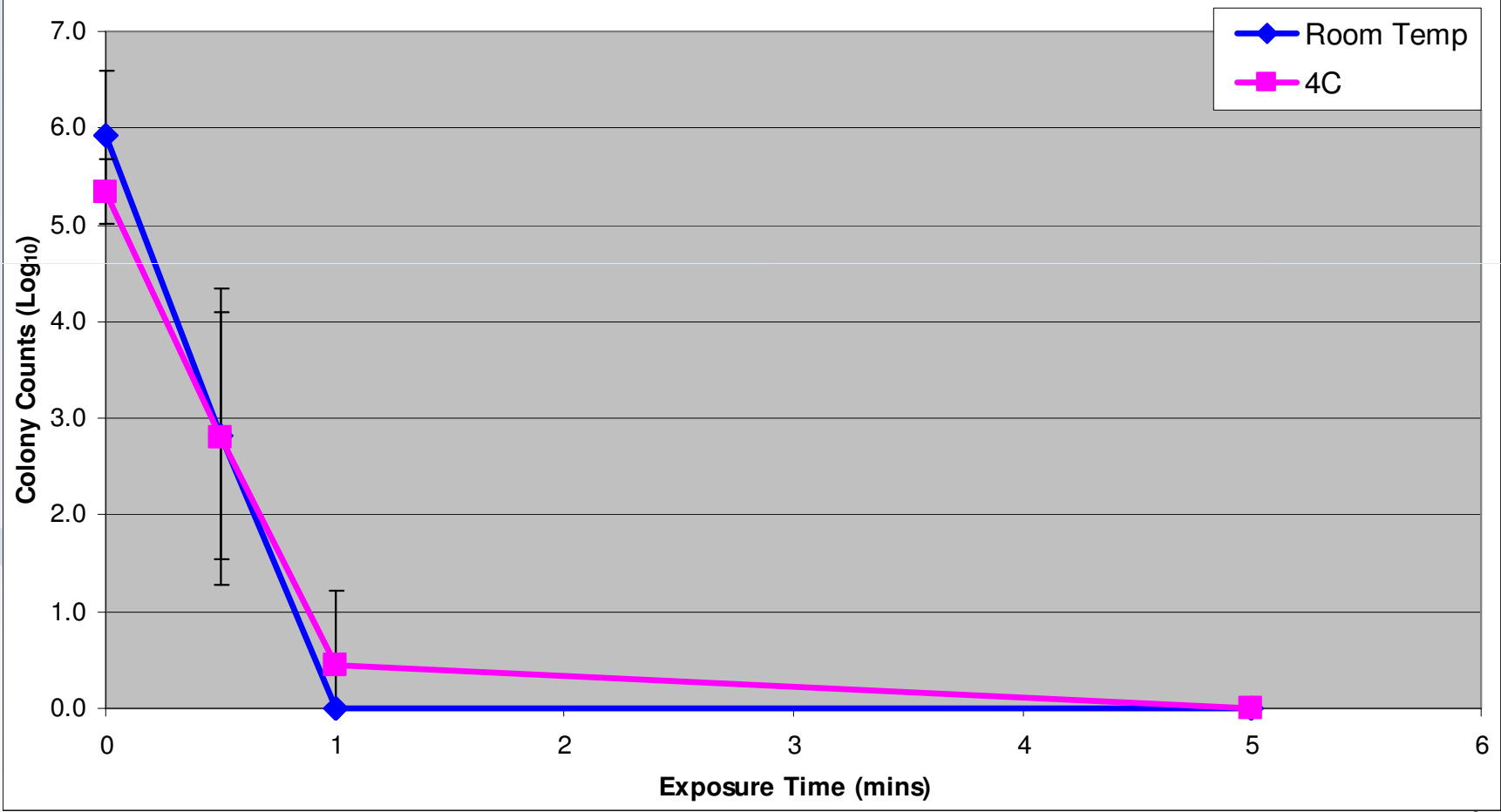


70% Ethanol



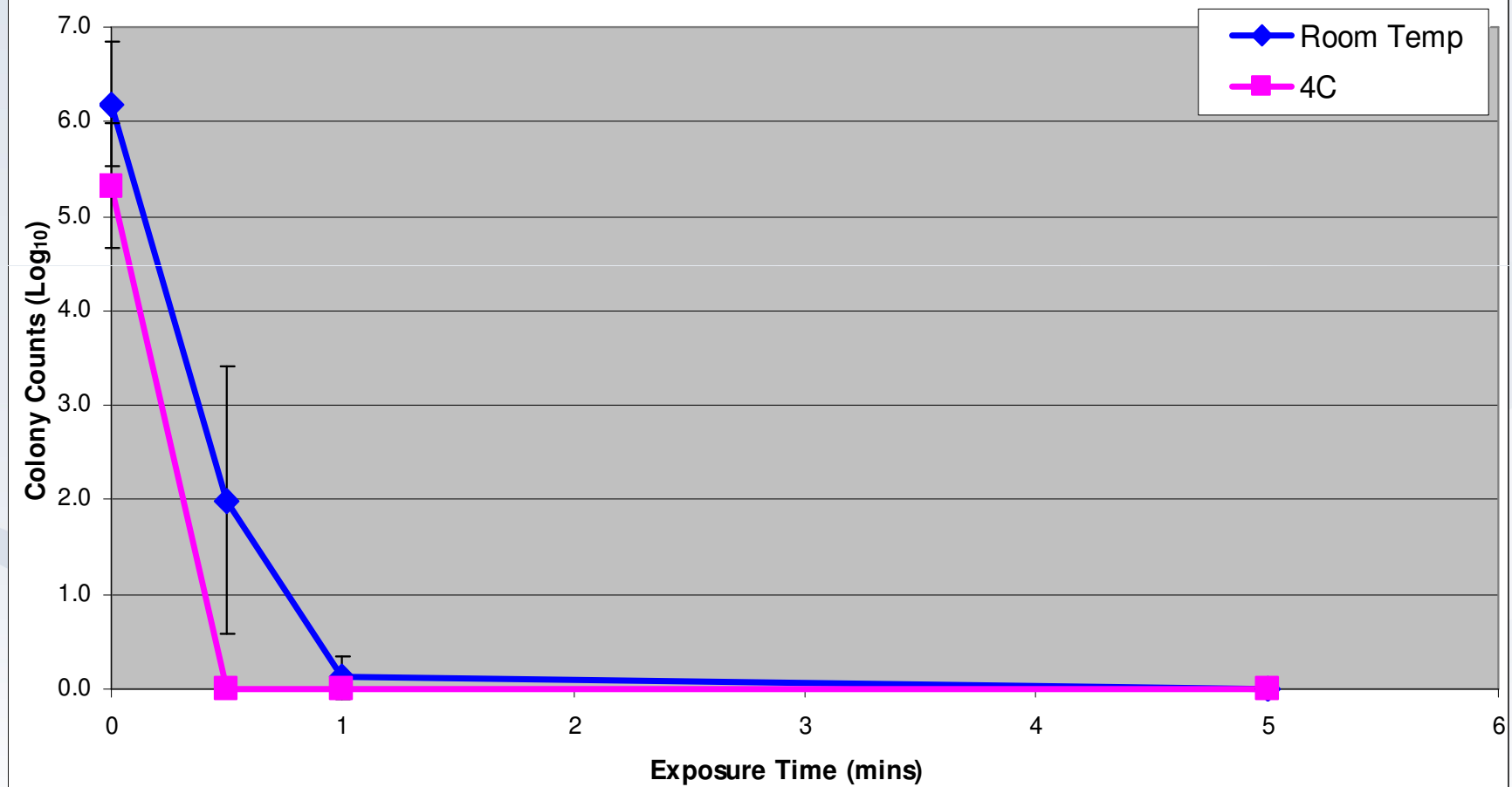
1/200 VigorOX

Comparison Between the Inactivation of *Listeria monocytogenes* at Room Temperature and 4C Using 1/200 Vigorox

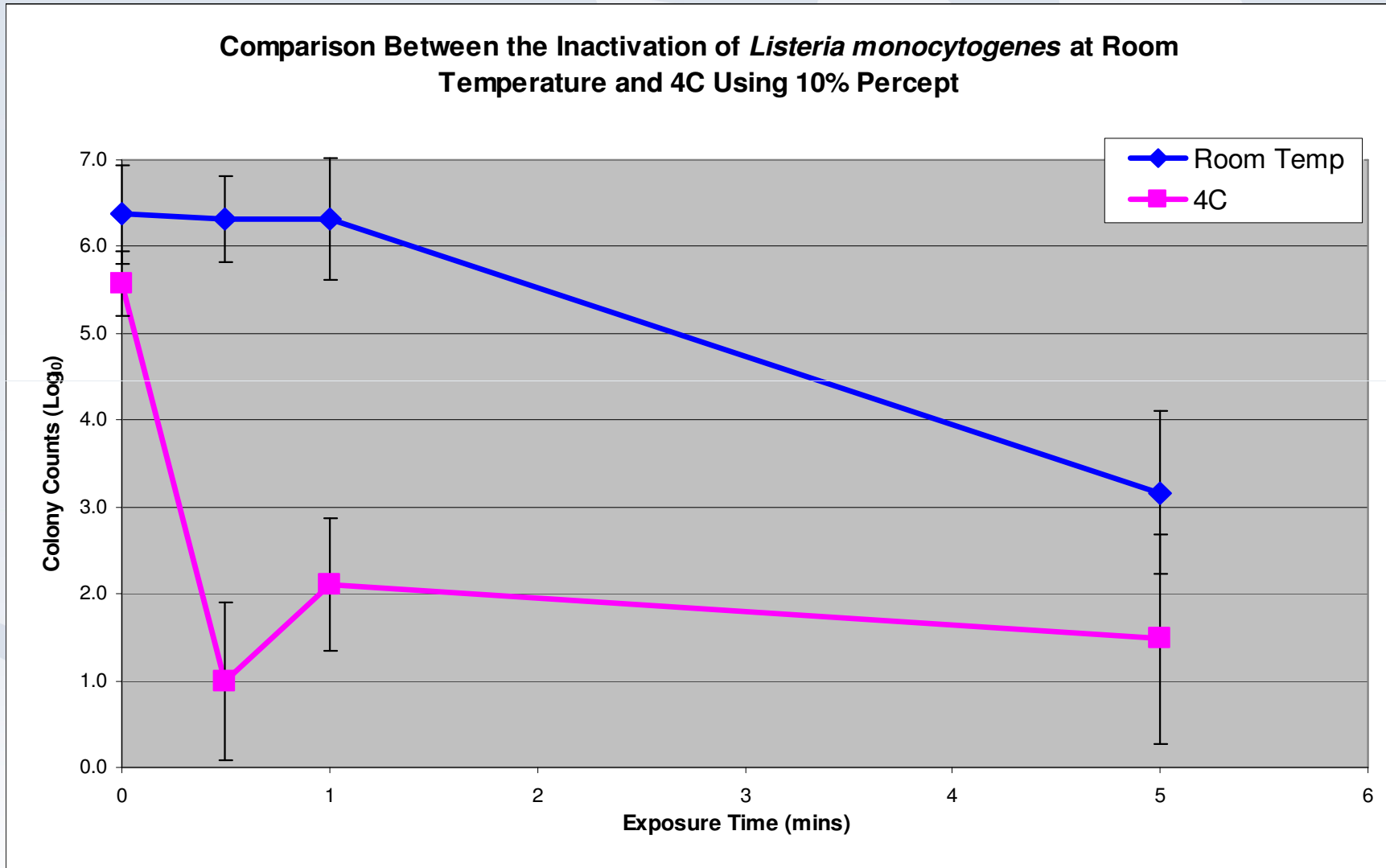


5% Bleach

Comparison Between the Inactivation of *Listeria monocytogenes* at Room Temperature and 4C Using 5% Bleach



10% Percept



Summary

| Disinfectant | D Value | | | | p Value (p<0.05) |
|---------------|---------|---------|-------|---------|---------------------|
| | RT | Std Dev | 4°C | Std Dev | |
| 10% Microchem | 1.088 | 0.156 | 1.008 | 0.409 | 0.388 |
| 70% Ethanol | 0.134 | 0.037 | 0.943 | 0.693 | 0.090 |
| 1/200 VigorOX | 0.171 | 0.021 | 1.018 | 0.229 | 0.011 |
| 5% Bleach | 0.709 | 0.625 | 0.099 | 0.005 | 0.073 |
| 10% Percept | 1.480 | 0.222 | 3.098 | 2.031 | 0.151 |

Important Findings

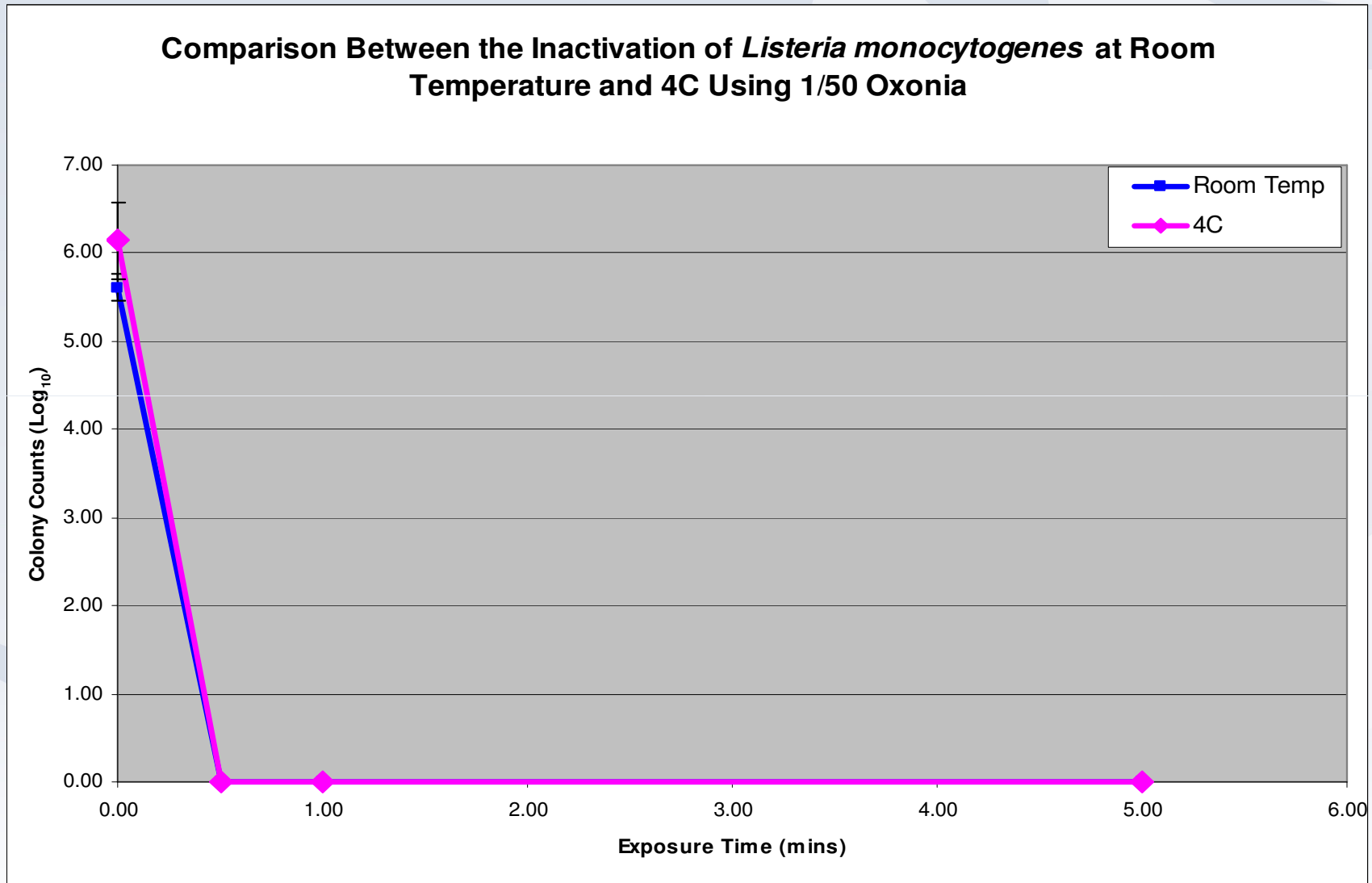
- Variation in performance of PAA (VigorOX) at RT and 4°C
- When tested at room and refrigeration temperature
 - Ethanol, bleach & PAA out performed
- Some disinfectants expected to work well did not (Virkon – data not shown)

Currently Used Disinfectants in the Canadian Food Processing Industry

- Oxonia Active (5.25% PAA, 24.15% hydrogen peroxide)
- Divosan Plus (PAA, hydrogen peroxide and acetic acid cpd)
- SU 393 (Isopropyl alcohol and lactic acid)
- Spectrum (Quaternary ammonium cpd)

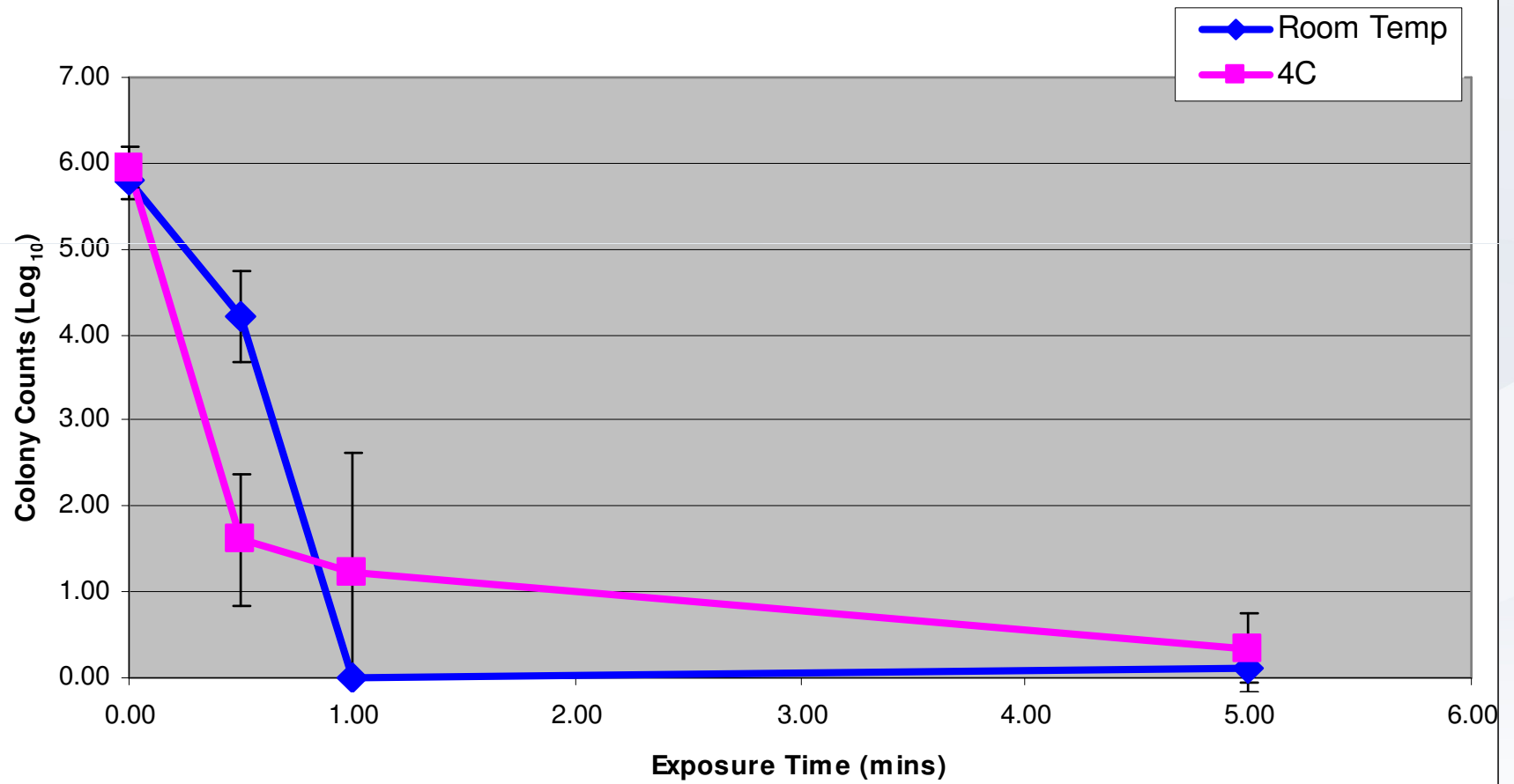


1/50 Oxonia



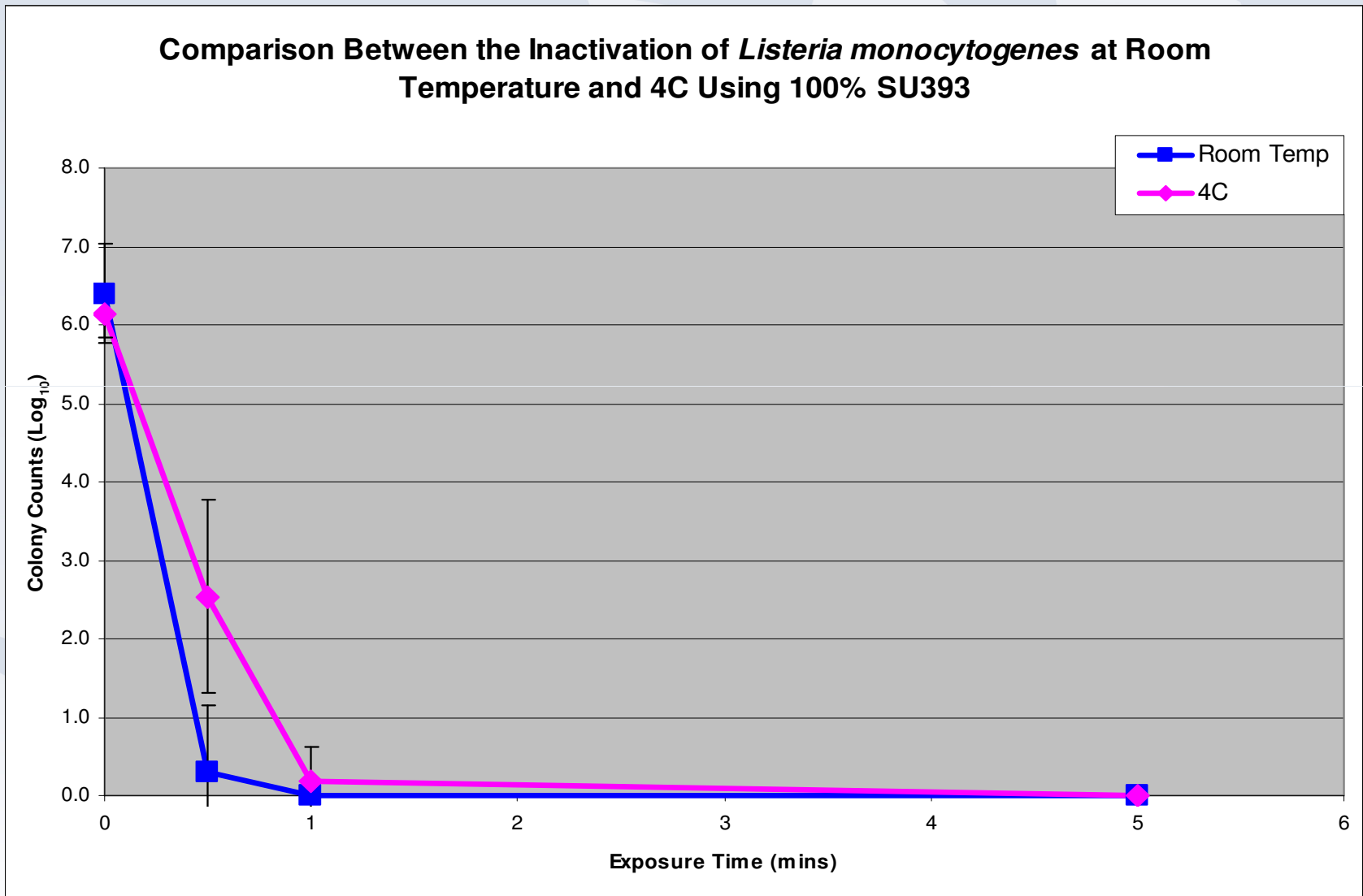
1/50 Divosan Plus

Preliminary Comparison Between the Inactivation of *Listeria monocytogenes* at Room Temperature and 4C Using 1/50 Divosan Plus



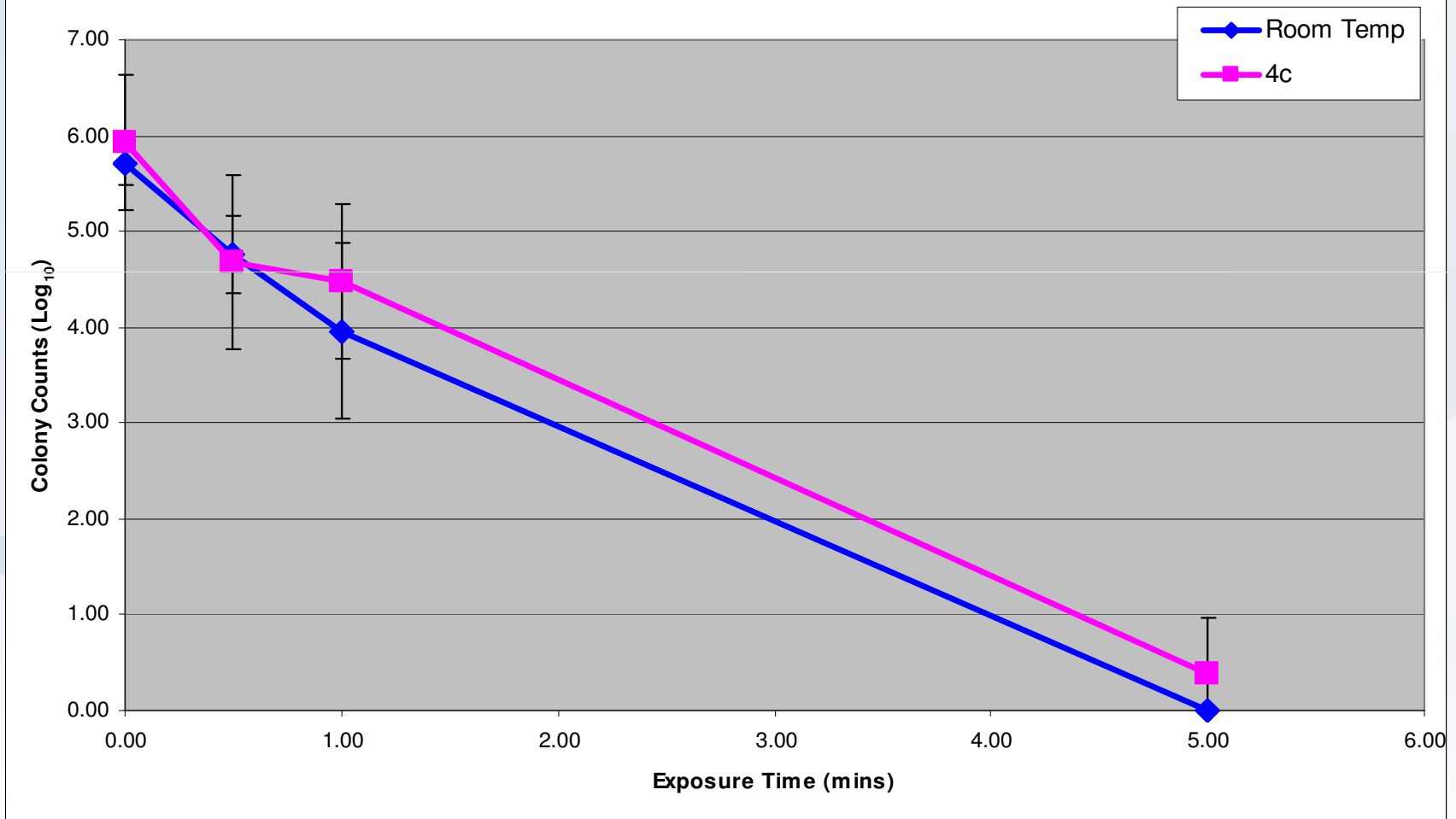
100% SU393

Comparison Between the Inactivation of *Listeria monocytogenes* at Room Temperature and 4C Using 100% SU393



10% Spectrum

Preliminary Comparison Between the Inactivation of *Listeria monocytogenes* at Room Temperature and 4C Using 10% Spectrum



Results

- Analysis of the data is currently unavailable as some results are preliminary
- Completion of testing is currently underway

Important Findings

- PAA & ethanol fit desired criteria best
 - Safe
 - Convenient
 - Low concentrations required
 - Fast
 - Effective at 4°C
- SU393 also very effective

Results From Current Studies

- Major Canadian food processing plants have changed decontamination procedures based on *Listeria* studies with the ABRP at NML
- No longer using QUATS – which were a major disinfectant employed by the plants
- Positive relations built between companies and laboratories – providing purpose to projects

Summary

- Variation in performance of disinfectants and the effect of different temperatures is very important when constructing decontamination procedures for food processing plants
- Standardized testing of disinfectants against organisms is essential in order to ensure safe practices and public health

Questions?