Validating Autoclave Cycles for Carcass Disposal in ABSL-2/3 Containment Laboratories

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Disclaimer

- This worked was sponsored by and conducted at the DHVI/Duke-RBL
- Sanofi Pasteur Limited was not involved in this work

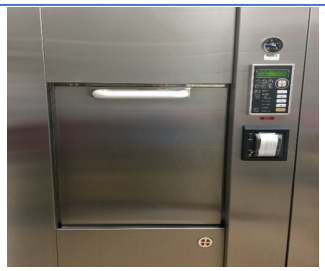
Background

- Infectious waste, including carcasses, needs to be sterilized before leaving the ABSL-3 facility for downstream disposal
- With start of ferret work, autoclave cycles need to be validated to ensure sterilization of potentially infectious carcasses

Background: Initial Results

- Standard cycle parameters used for solid biohazardous waste not sufficient for sterilizing intact ferret carcasses
  - Biological indicators (BIs) placed in and around carcasses were not inactivated

Cycle Parameters Used:
Time: 90 minutes
Temperature: 123.1^{o}C
Prevac: 15 PSI
# of Prevacs: 3



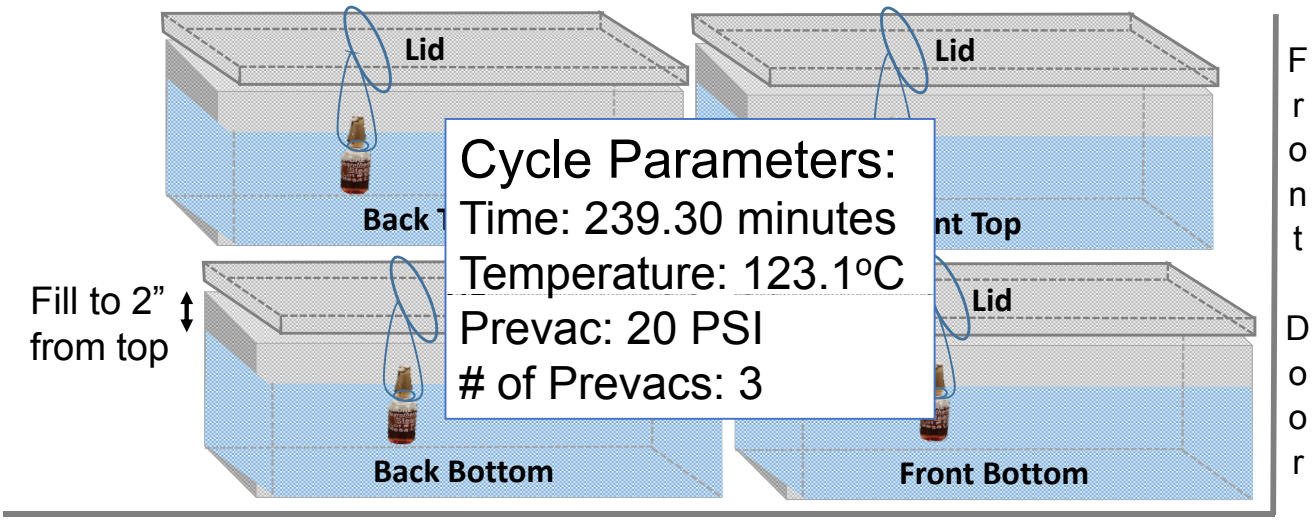
Objectives of Study

- To develop and validate autoclave cycle to ensure sterilization of small/medium-sized carcasses
- To develop a method for implanting BIs into intact carcasses
- To determine maximum carcass load per biohazard bag and number of bags per pan
- To determine total maximum load size/cycle

Experimental Design: Water Test

- Based on previous results:
  - Chose a 4-hour cycle
  - 123°C
  - 3 prevacs at 20 PSI
- Stainless steel pans used:
  - Durable, inexpensive, easy to clean
  - Filled with water
  - Placed biological indicators (BIs) inside pans

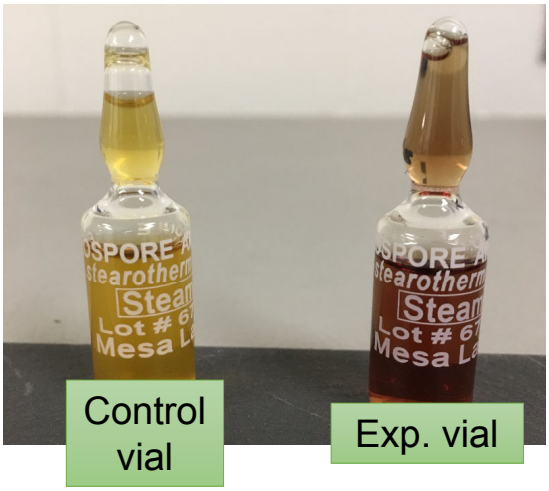
Experimental Design: Water Test



Cycle Parameters:
Time: 239.30 minutes
Temperature: 123.1°C
Prevac: 20 PSI
# of Prevacs: 3

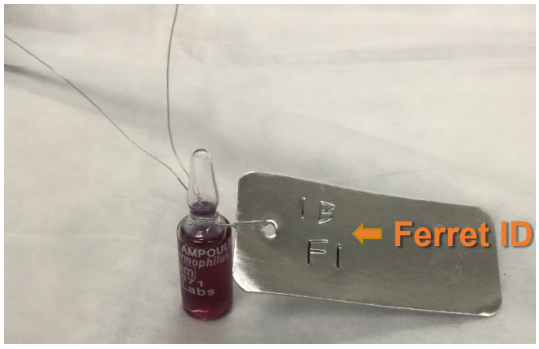
Results of Water Test

- All BIs passed (no growth)
- Next step: test cycle on ferret carcasses



Experimental Design: Ferret Test

G. stearothermophilus ampoule tied to label w/wire

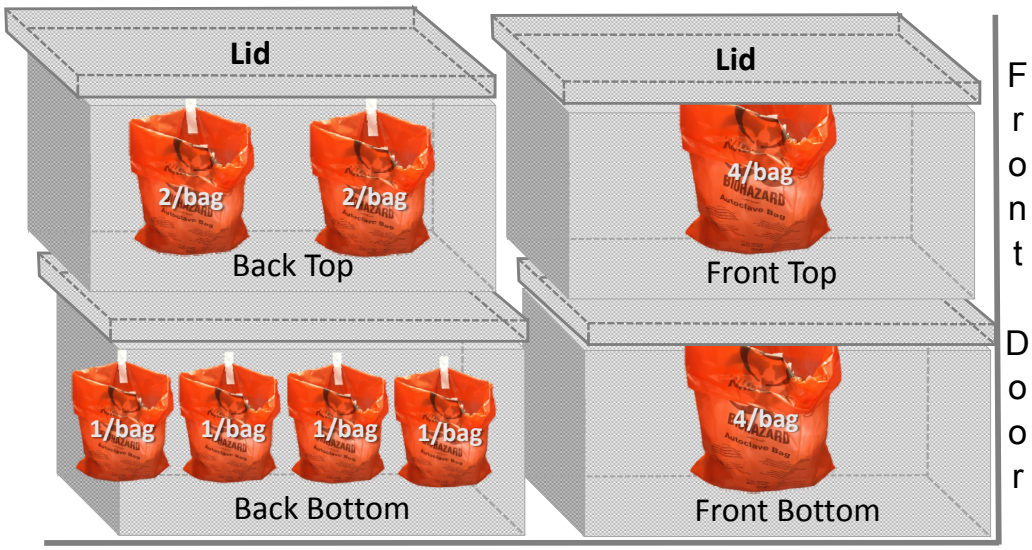


Ampoule placed in carcass via small incisions; wire affixed to spine

Experimental Design: Ferret Test

Each pan contains 4 ferret carcasses total (each weighing ~ 2kg)

Experimental Design: Ferret Test

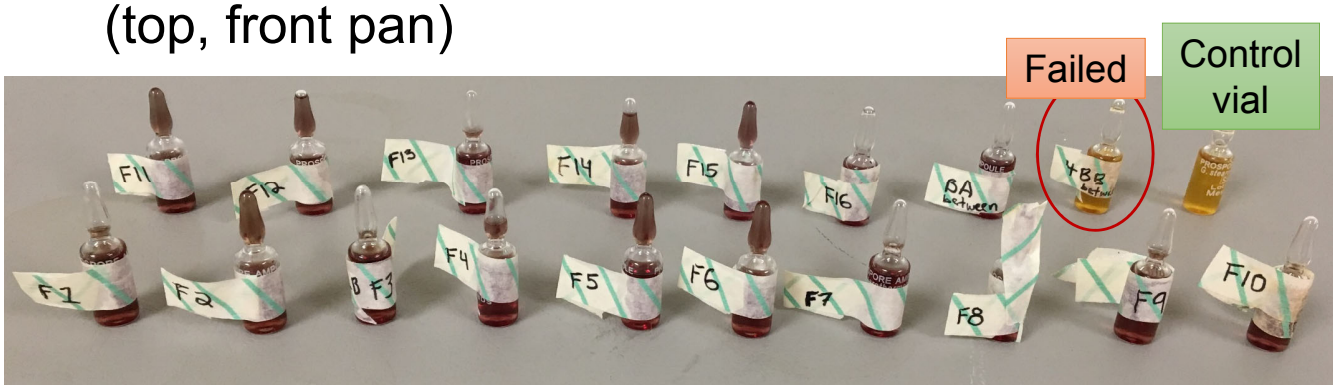


Experimental Design: Ferret Test

Cycle Parameters:
Time: 239.30 minutes
Temperature: 123.1°C
Prevac: 20 PSI
# of Prevacs: 3

Results

- All but one validation passed
- Vial placed in between carcasses failed (top, front pan)

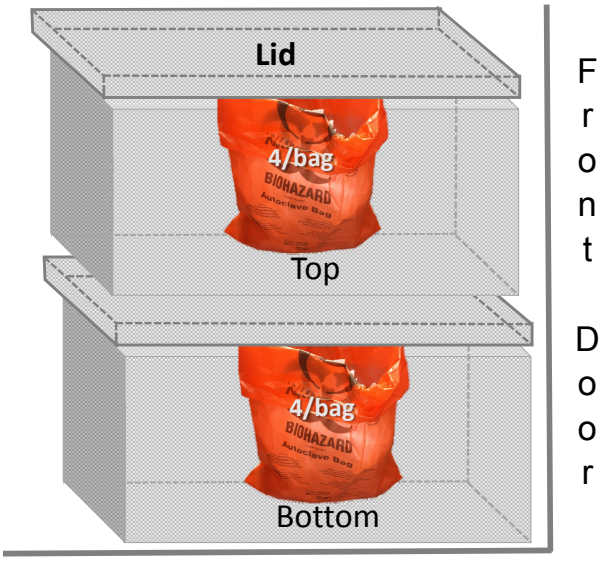


Results

Experimental Design: Modification

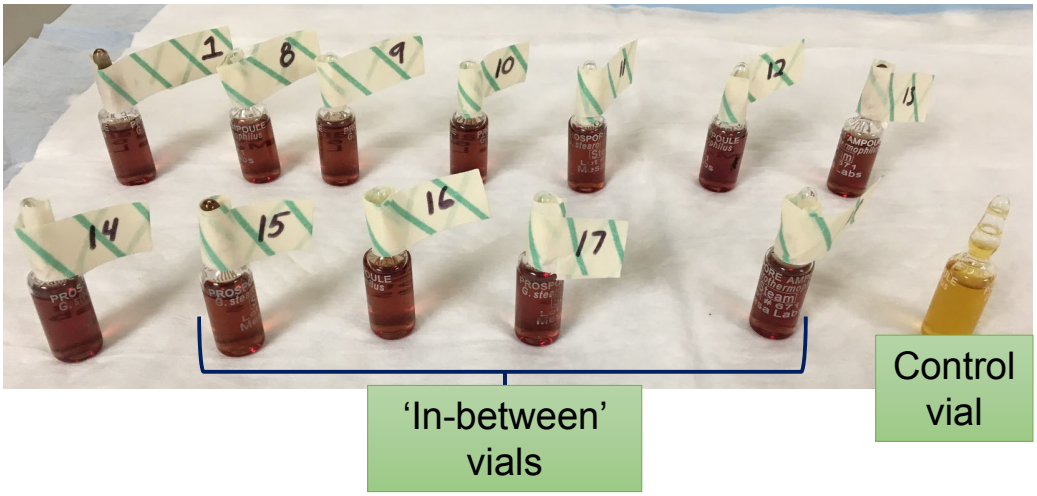
- Increase temperature
- Increase number of prevacs and lower PSI
- Used fresh or frozen carcasses

Cycle Parameters:
Time: 239.30 minutes
Temperature: **125°C** (123.1°C)
Prevac: **15 PSI** (20 PSI)
# of Prevacs: **5** (3)



Results

- All validations passed with either fresh or frozen carcasses



Conclusion

- Autoclave cycle time, temperature and number of pre-vacuum pulses is critical to ensuring sterilization of carcasses
- Sterilization was validated with up to 4 ferret carcasses/pan, 4 pans per cycle (16 total)
  - Multiple carcasses per load can be sterilized under validated autoclave conditions

Future Directions

- Repeat experiment to confirm results
- Increase number of pans per autoclave to 6 (24 carcasses total)
- Test with other types of carcasses (e.g., rabbits)

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Thank You!