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COMPARISON OF SPORE STRIPS & SELF-CONTAINED BIOLOGICAL INDICATORS FOR USE WITH FORMALDEHYDE DECONTAMINATION

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INTRODUCTION

Why was this study undertaken?

- To compare efficacy of rapid biological indicators with spore strips for area decontamination with formaldehyde
- Use of rapid biological indicators would be preferable due to ease of handling & reduction of incubation time




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INTRODUCTION

Formaldehyde decon used for:

- laboratories, animal cubicles, air locks
- biological safety cabinets
- HEPA filter housings
- buildings
- equipment compromised by steam or liquid
- field remediation

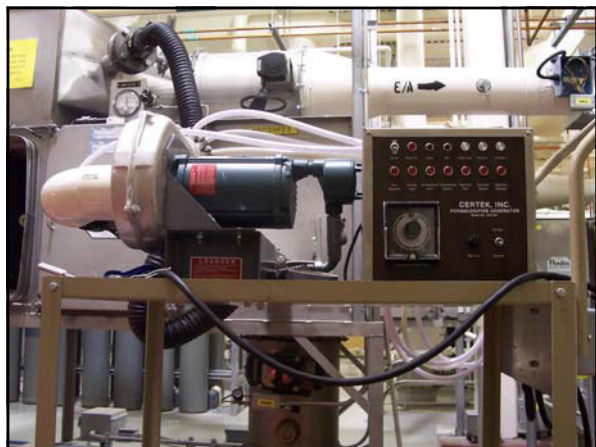


INTRODUCTION

- earliest reported - late 1880's
- used in laboratory, medical & industrial settings
- large scale:
 - poultry farms
 - Gruinard Island after *B. anthracis* spore experiments during WWII
 - mail sorting/stamping machine - US Dept of Justice








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INTRODUCTION

Formaldehyde decontamination process:

- **Decon:** 0.3g paraformaldehyde / ft³
- **Neutralization:** ammonium carbonate (g)
= paraformaldehyde (g) X 1.2
- > 70% relative humidity
- 6, 8, 10, 12+ hours decon
- decontamination of rooms, animal cubicles, in-line HEPA housings, equipment




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INTRODUCTION



Preliminary studies on:

- 1) Spore strips**
 - *Bacillus atrophaeus*
 - *Geobacillus stearothermophilus*
- 2) Self-contained biological indicators (48h)**
- 3) Enzyme-based biological indicators**
 - 3h enzymatic reaction
 - 48h growth



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BIOLOGICAL INDICATORS

- 1) Spore strips**
 - 'gold standard' biological indicators
 - filter paper strip impregnated with spores contained in glassine envelope
 - *B. atrophaeus* or *G. stearothermophilus*
 - usually 10⁶ organisms/strip
 - can be used with glassine envelope closed (manuf. claim)


BIOLOGICAL INDICATORS

1) Spore strips (cont'd)

- must have a lab at your disposal
- must have laminar flow hood or BSC
- must have person trained in aseptic technique to transfer/grow
- results in 3 to 7 days
- not user-friendly



BIOLOGICAL INDICATORS

2) Self-contained biological indicators (48h)

Equipment needed:

- self-contained biological indicator
- dedicated heating block incubator
- quicker results & easy to use



BIOLOGICAL INDICATORS

2) Self-contained biological indicators (48h) (cont'd)

- consist of paper strip inoculated with spores packaged in vial along with growth medium



BIOLOGICAL INDICATORS

3) Enzyme-based biological indicators (3h)

Equipment needed:

- self-contained biological indicators
- dedicated heating block incubator



BIOLOGICAL INDICATORS

3) Enzyme-based biological indicators (3h) (cont'd)

- third generation biological indicator system
- detects alpha-D-glucosidase upon growth of spores (3h)



BIOLOGICAL INDICATORS

3) Enzyme-based biological indicators (3h) (cont'd)

- bacterial growth detected in 48 hours
- quicker & easier to use than spore strips



TEST SITES

1) Decontamination room / animal cubicles

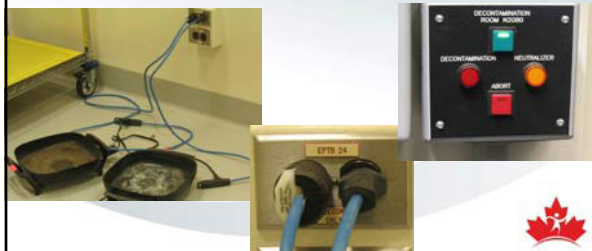
- sealed rooms with submarine doors



TEST SITES

1) Decontamination room/animal cubicles (cont'd)

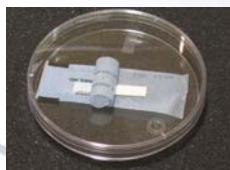
- decon carried out using electric skillets



TEST SITES

1) Decontamination room/animal cubicles (cont'd)

- 3 types of indicators used



TEST SITES

2) In-line HEPA housing

- sealed 'container' with bubble-tight dampers



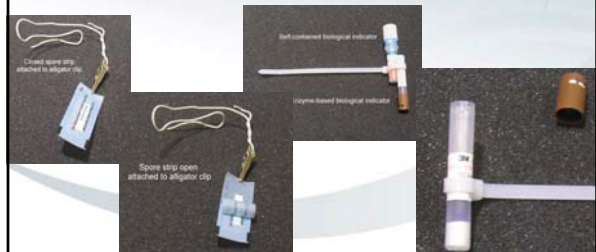




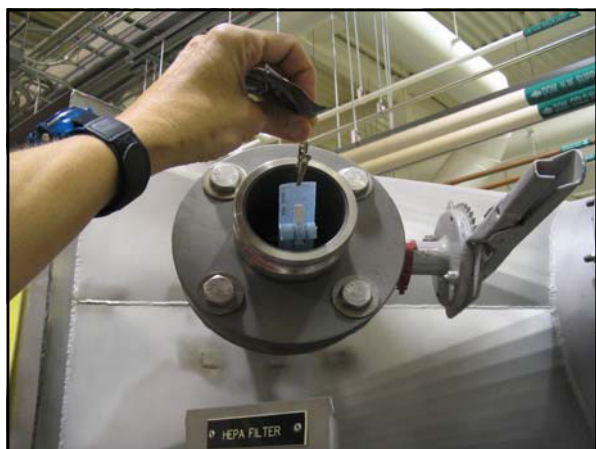
TEST SITES

2) In-line HEPA housing (cont'd)

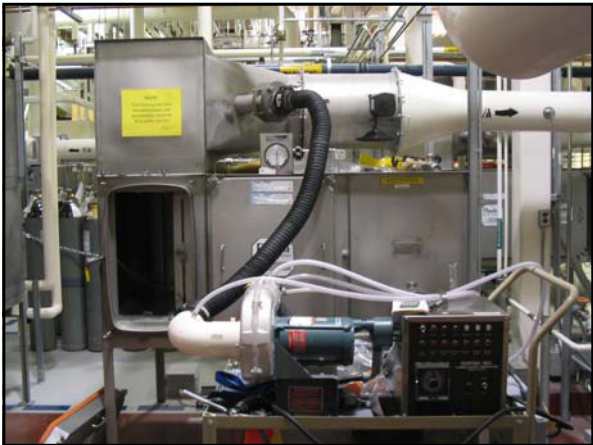
- 3 types of indicators used - open or closed

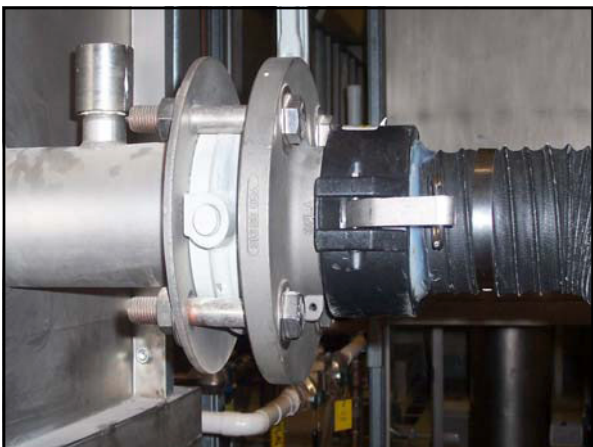














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METHOD

1. Spore strips

Sealed glassine envelope Open glassine envelope

Spore strips

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METHOD

1. Spore strips (cont'd)

To grow - transferred to 6.5ml TSB, and

- incubated at 37°C (*B. atrophaeus*)
- incubated at 55°C (*G. stearothermophilus*)

Positive spore strip Negative spore strip

METHOD

2. Self-contained biological indicators (48h)

- incubated at 37°C as per manufacturers instructions
- incubated at 55°C as per manufacturers instruction
- incubated at 37°C with the spore strip placed in 6.5 ml TSB



METHOD

3. Enzyme-based biological indicators

- incubated at 60°C as per manufacturers instructions (top **on** during decon)



METHOD

3. Enzyme-based biological indicators (cont'd)

- incubated at 60°C as per manufacturers instructions (top **off** during decon)



METHOD

3. Enzyme-based biological indicators (cont'd)

- results read at 3 hours (enzymatic)



METHOD

3. Enzyme-based biological indicators (cont'd)

- results read at 48 hours (growth)



METHOD

Formaldehyde decontamination times:

- 6 hours
- 8 hours
- 10 hours
- 12 hours +



Comparison of Spore Strips, Self-contained and Enzyme-based Biological Indicators (pos/total)


Formaldehyde Hold Time	Spore Strips				Self-contained Indicators	Enzyme-based indicators	
	B. atrophaeus		G. stearothermophilus			48 hours	3 hours
	sealed	open	sealed	open			
6 hours	6/6	1/6	5/6	1/6	3/49	13/94	0/72
8 hours	9/9	0/13	9/9	2/13	0/25	12/28	0/24
10 hours	2/2	0/4	4/4	0/4	0/18	0/6	0/12
12 + hours	1/10	0/6	1/9	0/10	0/10	0/6	0/4

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DISCUSSION

SPORE STRIPS

- results similar for both types of spore strips for all decon times (open and closed)
- for decon < 12 hours, glassine envelope must be open
- not user-friendly




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DISCUSSION

SELF-CONTAINED BIOLOGICAL INDICATORS (48 h)

- work well with 8 to 12 hour decon
- no apparent false negatives
- no difference in results for bacteria selected
- convenient and easy to use




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DISCUSSION

ENZYME-BASED BIOLOGICAL INDICATORS (3h)


- 1) 3 hour enzymatic indicator
 - works best > 10 hours decon
- 2) 48 hours growth indicator
 - works well with 6 to 12+ hour decon
 - convenient and easy to use



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DISCUSSION

1. Spore Strips (7d)
 - should be 'open' for decon; not user friendly
2. Self-contained Biological Indicators (48h)
 - work well for 8 -12h decons; quick & easy
3. Enzyme-based Biological Indicators (3h, 48h)
 - **3h:** best results with >10h decon
 - **48h:** work well for 6-12h+ decon; quick & easy




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CONCLUSION

Preliminary studies indicate that formaldehyde decontaminations of > 8h, using self-contained biological indicators (48h growth):

- ✓ show comparable or better results than spore strips
- ✓ are reliable and easy to use
- ✓ reduce the decon down-time of areas to 48h from up to 7 days





Thank you!
