Decontamination of Biological Agents from the Surface of Materials of Military Importance

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Outline

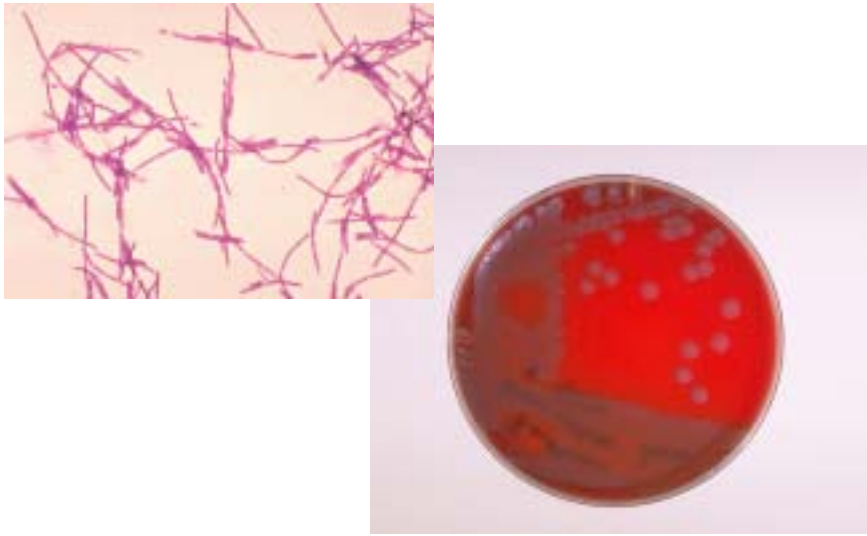
- Objectives
- Requirements & Guidelines
- Test Challenges
- Procedures
- Results & Conclusions
- Future Issues

Objectives

- **Develop method to determine the efficacy of candidate decontaminants on surfaces of military importance**
- **Begin to bridge the gap between laboratory testing and 'real world' application**

Requirements & Guidelines

- Utilize decontamination procedures in FM 3-5 as a guideline, modifying for the evaluation of decontaminants
- Provide quantitative assessment of decontaminant(s) efficacy



Requirements & Guidelines

Test Layout

- Surfaces
  - Sand, soil, concrete, asphalt
    - ~1 cm^2 surface area
    - Approximately 1 inch (2.54 cm) deep
- Challenge levels
  - *Bacillus anthracis* spores: 10^6 spores/cm^2 (JSFDS TEMP: Table 1-6)
- Replicates: Five
- Decontaminant amounts (FM 3-5)
  - Based on amount of decontaminant required for 10 g/m^2 chemical agent challenge (50:1 w/w)

Requirements & Guidelines

Test Layout (cont.)

- Allow contamination to remain 60 min prior to decontamination (NATO requirements AEP 7)

- Decontaminant contact time: 30 min. (FM 3-5)

Test Challenges

- Agent application to material surface
- Decontaminant application to the surface
- Agent recoverability from the surface
- Distinguish between bacteriostatic and bacteriocidal effect
- Reduction of background contamination on surfaces
- Effective quench/neutralization of decontaminant after 30 minutes

Procedure

- Select appropriate method to quench decontaminant
  - Example: For oxidizers, consider a reducing agent, such as sodium metabisulfite or sodium thiosulfate
  - Determine appropriate concentration of neutralization solution
    - Use an excess of neutralizer based upon molar ratios
    - Assay solution to verify absence of active component after neutralization
- Neutralize decontaminant and test on agent
  - Demonstrate minimal effect of neutralized decontaminant on viability of biological agent
  - Demonstrate recoverability of spores from surfaces

Procedure

Eliminate background contaminants from the surfaces

Spiked and untreated controls were tested

Asphalt & Concrete

- Boiled for 5 minutes
- Dried at 120 °C in dry convection oven
  - Asphalt – 2 hours
  - Concrete – 1 hour

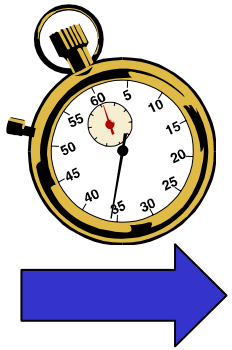
Soil & Sand

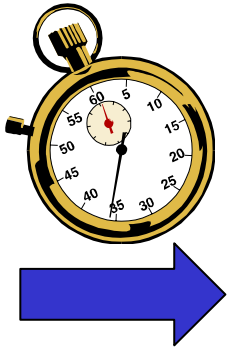
- Autoclaved 60 minutes at 132°C , 28.5 psi, on a dry cycle
- Dried for 2 hours at 120 °C in dry convection oven

Procedure

Add Agent **Add Decontaminant** **Neutralize**

60 minutes **30 minutes**





Procedure

Add Media

- Final volume = 5 ml

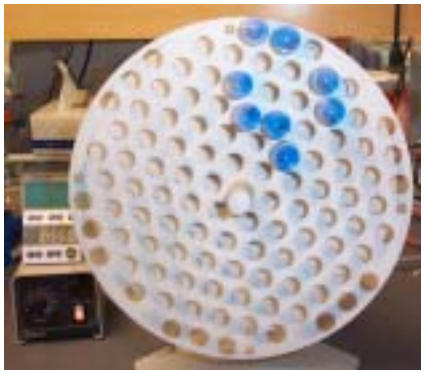
Vortex

- Sand & soil

or

Rotating Shaker

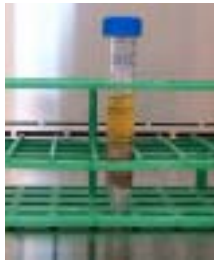
- Concrete & asphalt



Viability Assay

Procedure

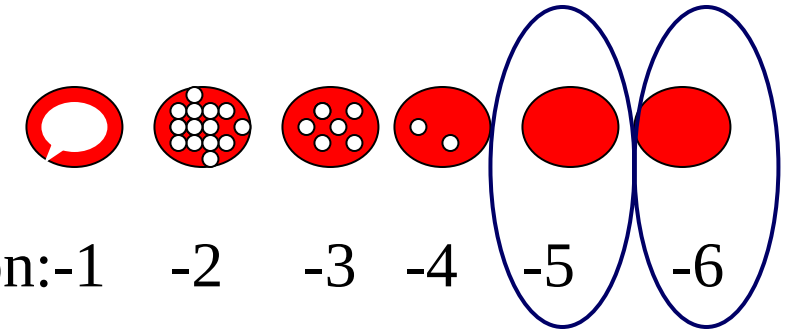
- Serial dilutions from each sample
  - Perform dilutions in media (900 µl media per tube)
- Plate 100 µl from each dilution tube, in duplicate
- Incubate at 37°C
  - Plates – 48 hours
  - Dilution tubes – up to 20 days (determined by test deadline)
  - Sample containing material - incubate with shaking



Procedure

Determine Efficacy of Decontaminant

- Count colony forming units
- Select highest two dilutions where zero growth is observed
  - Pipette remaining volume in each corresponding dilution tube into fresh 25 ml of media
  - Allow tubes to incubate in shaker/incubator for 48 hrs
  - Subsample tubes and plate in duplicate on appropriate media
  - Incubate plates and count colony forming units



Results & Conclusions

- To date, we have tested this procedure on:
  - Peroxygen-based decontaminants
    - Alkaline
    - Acidic
  - Hypochlorite-based decontaminants
- Recoverability of biological agents from surfaces treated with *neutralized* decontaminant
  - Not significantly different from untreated surfaces ($p<0.05$)
  - Minimal variability within replicates (<0.5 log)
- Decontaminant efficacy results were consistent (minimal variability) for plastic and stainless steel substrates
- Variable decontaminating results were observed (>2 log difference) when asphalt, concrete, soil or sand surfaces were treated with *active* decontaminant

Future Issues

- Porous surfaces (asphalt, concrete, soil, sand, etc.)
  - Interaction between biological agent and decontaminant
  - Interaction between decontaminant and surface materials
  - Aggregation of spores
- Application of agent and decontaminant
- Number of days to incubate dilution tubes
- Media selection
- Standardization of procedures
- Validation

Acknowledgements

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Backup Slides

References

NATO References

Quadripartite Standardization Agreements (QSTAG), Standard 747, Edition 2, AEP-7. *NBC Survivability Acceptance Criteria, Design Guidelines, and Test Procedures for Defense Equipment Decontamination Survivability Criteria for Military Equipment*, Section II. Acceptance Criteria.

FM 3-5 References

Decontamination Stations

"Detailed Equipment Decon" section

- Pages 4-18 through 4-22
- Pages 4-19 through 4-23 (Change 1, 31 Jan 02)

References

Calculation of Decontaminant Amount

$(10g\ agent/m^2) \times (50g\ decon/g\ agent) = 500g\ decon/m^2$

$(500g\ decon/m^2) \times (m^2/10^4\ cm^2) = 0.05g\ decon/cm^2$