The Sporicidal Activity of Residual Formaldehyde Absorbed onto Paper Strips of Biological Indicators

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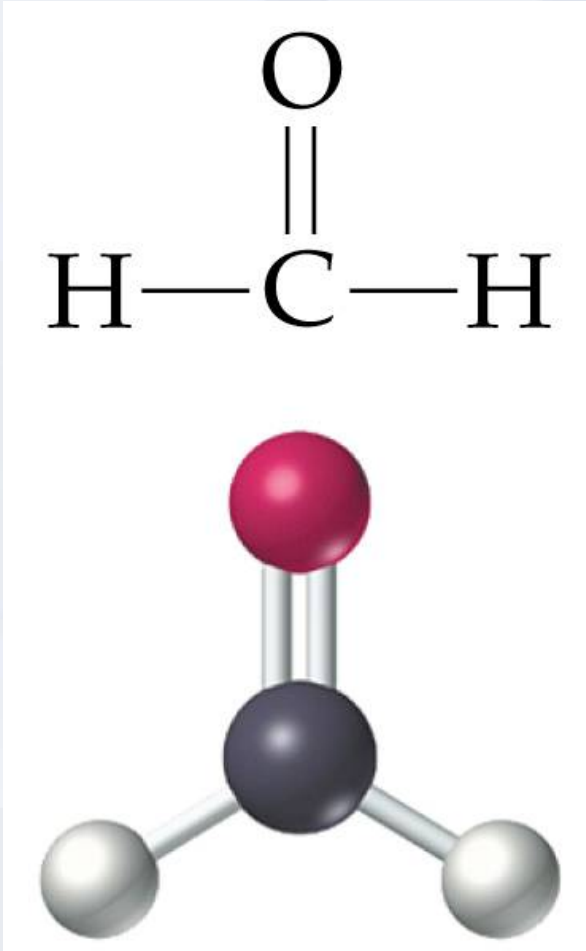
Introduction: Formaldehyde

- Colourless gas with pungent odour
- Flammable, combustible and corrosive
- Irritant to skin, eyes and respiratory system
- Known human carcinogen (International Agency for Research on Cancer)



History of Formaldehyde

- First synthesized by a Russian chemist in the early 1880s
- Recognized for its bactericidal properties by Loew in 1886
- Used as a vapour phase decontaminant since 1890s

Applications

- Efficient and economical decontamination in laboratory and medical settings
  - Biosafety Cabinets
  - Animal cubicles
  - HEPA filter housing units
  - Containment laboratories
- Large Scale
  - Buildings affected by bioterrorism

Decontamination Procedure

- Depolymerization of paraformaldehyde:
  - Forms formaldehyde gas when heated to 232-246°C
- Neutralization of formaldehyde
  - Ammonium carbonate
  - Produces hexamethylene tetramine, white powder residue on surfaces

Decontamination Procedure

Decontamination Procedure

Formaldehyde Pros and Cons

Pros:

- Widely accepted due to long time use and familiarity
- Efficiently inactivates a broad spectrum of organisms
- Economical

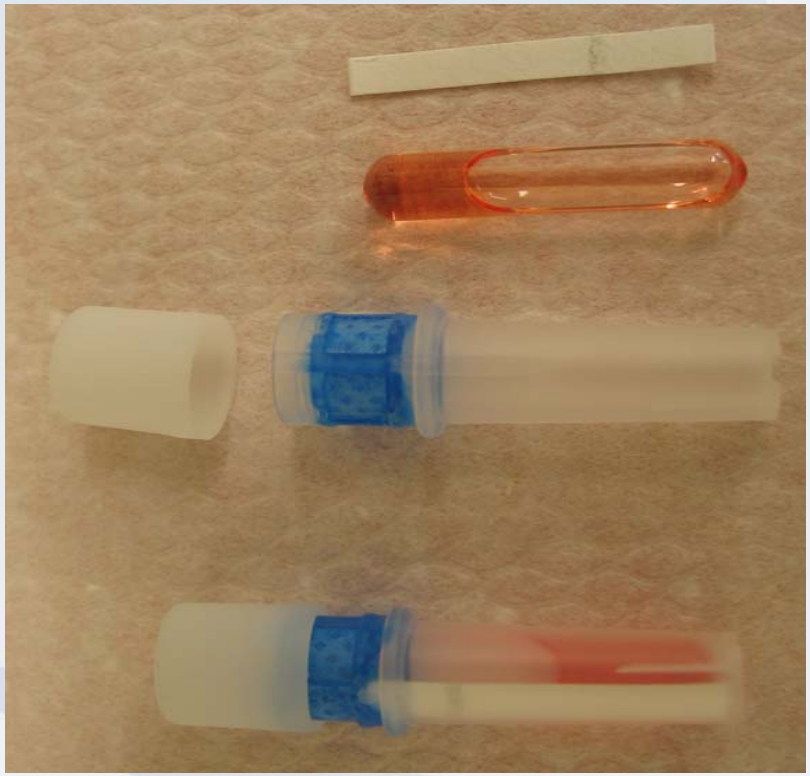
Cons:

- Probable human carcinogen
- Neutralization forms white residue which must be cleaned after decontamination

Biological Indicators- Spore Strip

- "Gold Standard"
- *Bacillus atropheaus* and *Geobacillus stearothermophilus* spores on a filter paper strip
- Packaged in a glassine envelope
- Envelope not permeable to formaldehyde

Biological Indicators- Self Contained



- Paper strip containing *Bacillus atropheaus* and *Geobacillus sterothermophilus* spores
- Glass vial of media
- Packaged in a plastic tube

Absorption of Formaldehyde by Surfaces

- Formaldehyde is absorbed on various surfaces during gaseous decontamination
- Highest levels of absorbed formaldehyde were found on cotton cloth
- Published in 1970 but overlooked in the design of biological indicators used for formaldehyde decontamination

Braswell *et al.* Applied Microbiology Nov 1970

Biological Indicators and Absorption of Fumigants

- Previous studies on Vaporous Hydrogen Peroxide (VHP) and BIs
  - Paper strip BIs absorb VHP
  - Stainless steel BIs do not absorb VHP

Absorption of Formaldehyde

- Due to absorptive properties of paper
  - Formaldehyde could be trapped in paper during decon
  - May inhibit growth of surviving spores

May lead to false negatives !!!!!

Objective

- Are detectable amounts of formaldehyde residue present in exposed paper spore strips?
- Are these formaldehyde residues inhibitory to the growth of spores?

Exposure to Formaldehyde

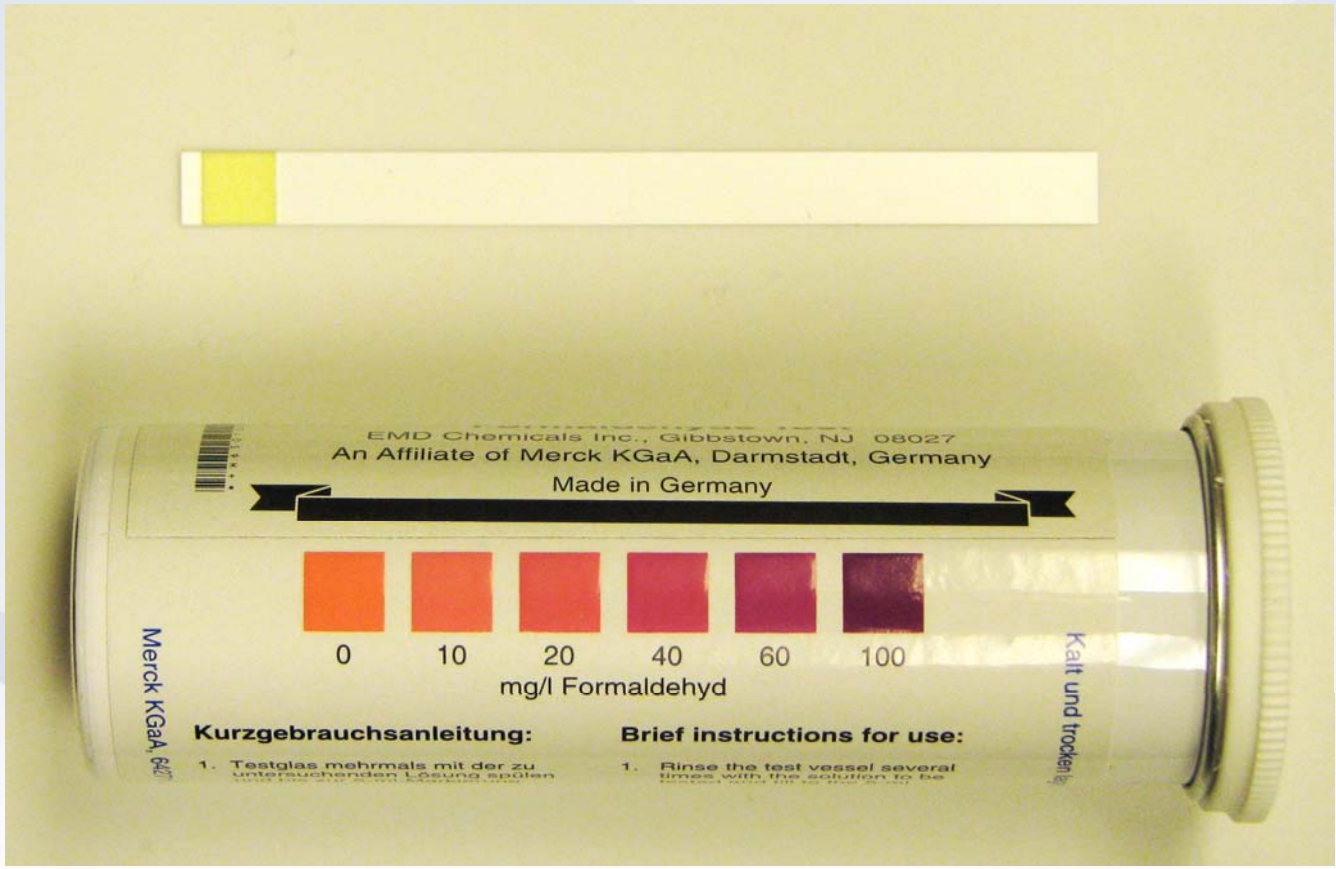
- Biological indicators placed at various locations within an IN-LINE HEPA housing
- Formaldehyde gas decontamination
  - Humidification
    - 70-90% Relative Humidity
  - Vaporization of paraformaldehyde
    - 0.3g/cft (approx 8000-10 000 ppm)
    - Minimum 8 hours exposure
    - Neutralization with ammonium carbonate

Testing For Residual Formaldehyde

- Paper spore strip is vortexed in water to elute residual formaldehyde
- Condensation of aldehydes with 4-amino-3-hydrazino-5-mercapto 1,2,3 triazol
- Atmospheric oxidation yields purple coloured tetrazine derivative

Testing for Residual Formaldehyde

Qualitative

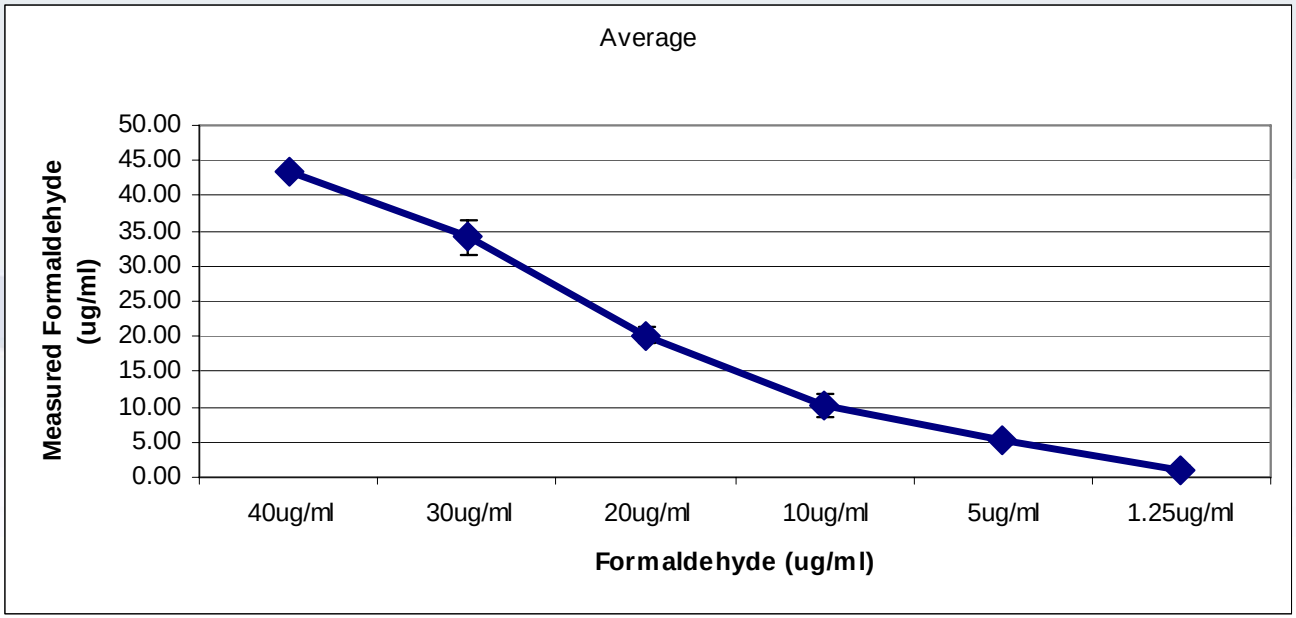


Testing for Residual Formaldehyde

Quantitative

RQFlex 10 Reflectometer

Dilution (ug/ml)	Average Reading (ug/ml)	Standard Deviation
40	45.53	2.50
30	34.13	1.25
20	20.19	1.71
10	10.10	0.53
5	5.10	0.36
1.25	1.13	0.15



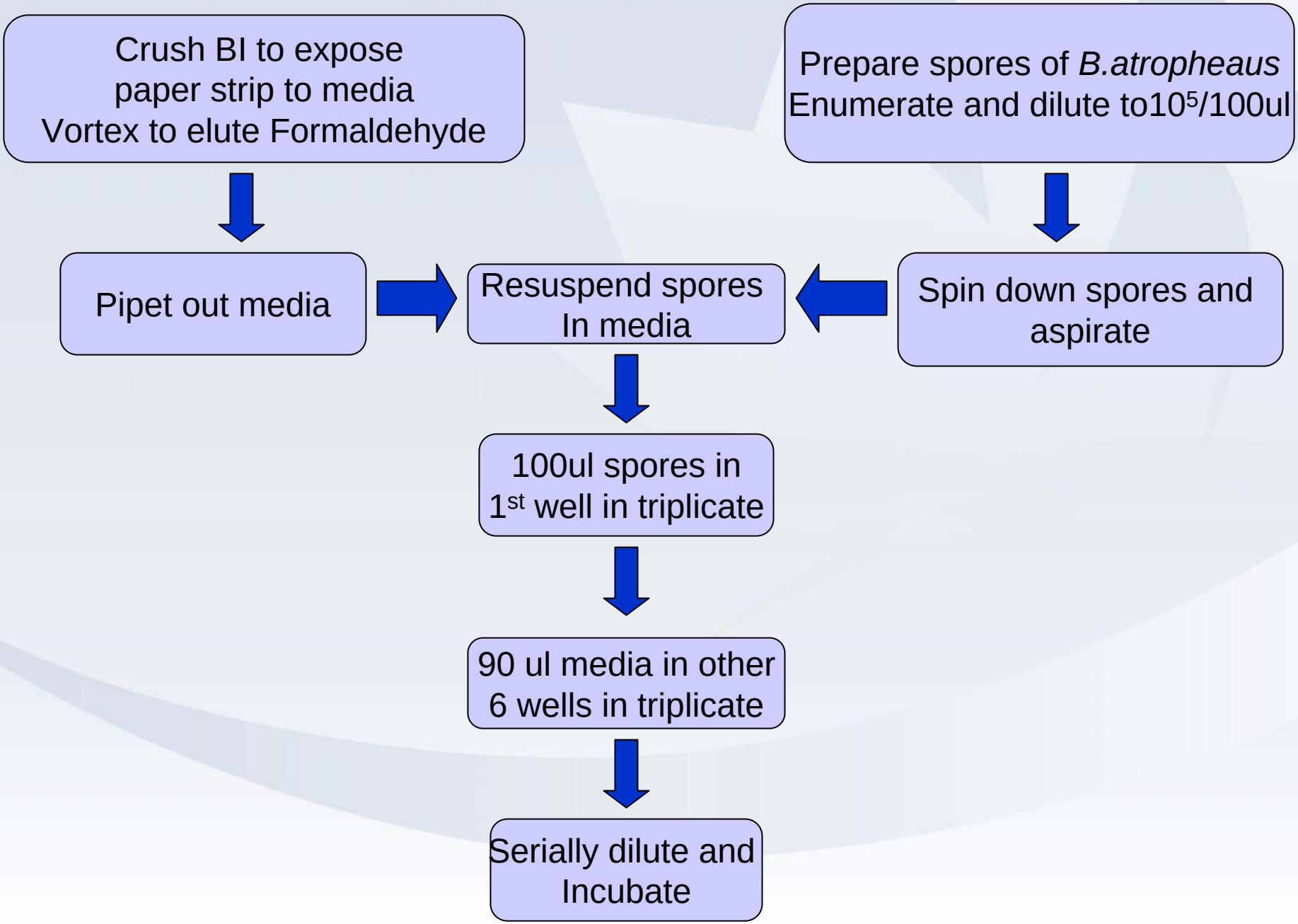
Testing for Residual Formaldehyde

Residual formaldehyde Concentration

- >50 BI with paper strips tested
  - Residual formaldehyde present in all concentrations range 1.3 to >45 mg/l

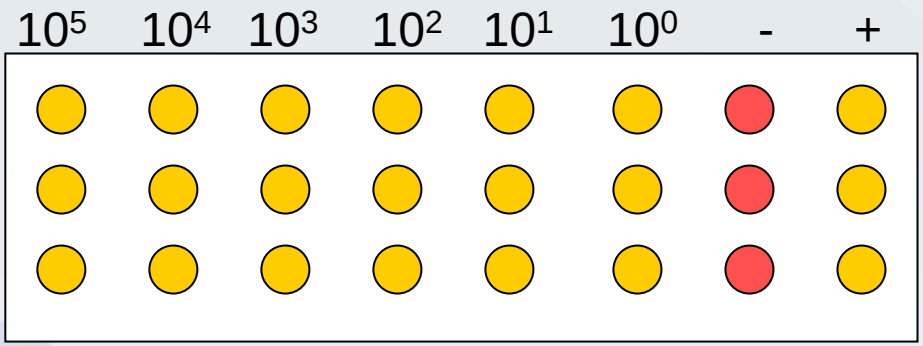
Testing for Inhibition of Growth

- Residual formaldehyde was eluted in the media inside the biological indicator
- This media was then used to resuspend spores of *Bacillus atropheaus* ATCC 9372
- These spores were diluted using the same media to test for inhibition of growth against different concentrations of spores from 100,000 to 1 spore

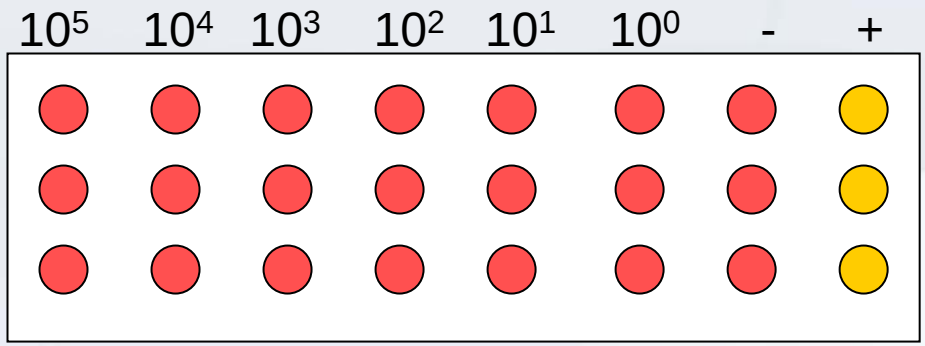

Crush BI to expose
paper strip to media
Vortex to elute Formaldehyde
Prepare spores of *B.atropheaus*
Enumerate and dilute to10^5/100ul
Pipet out media
Resuspend spores
In media
Spin down spores and
aspirate
100ul spores in
1st well in triplicate
90 ul media in other
6 wells in triplicate
Serially dilute and
Incubate

Testing for Inhibition of Growth

- 23 paper spore strip indicators were tested in triplicate
  - 21 inhibited growth of spores
  - Incubated for 7 days



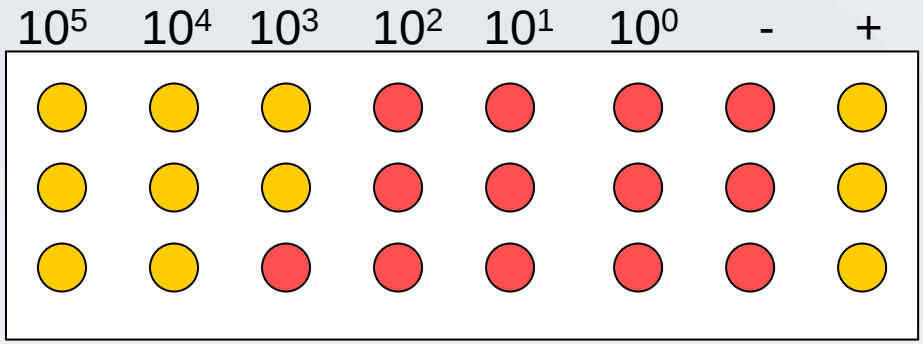
Control



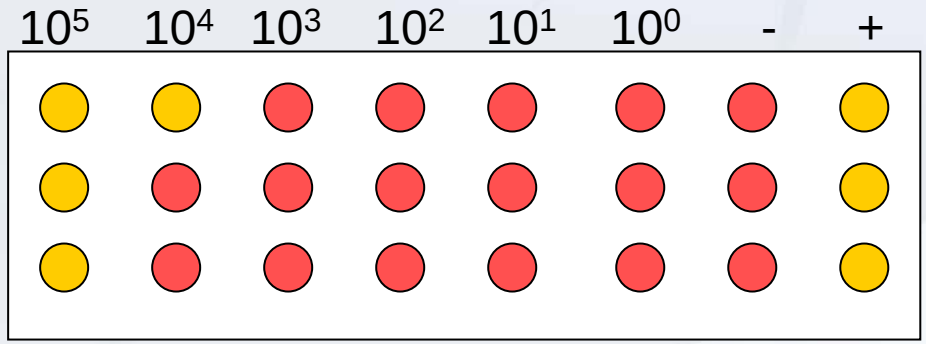
Test

Testing for Inhibition of Growth

- 2 out of 23 paper spore strip indicators did not show complete growth inhibition

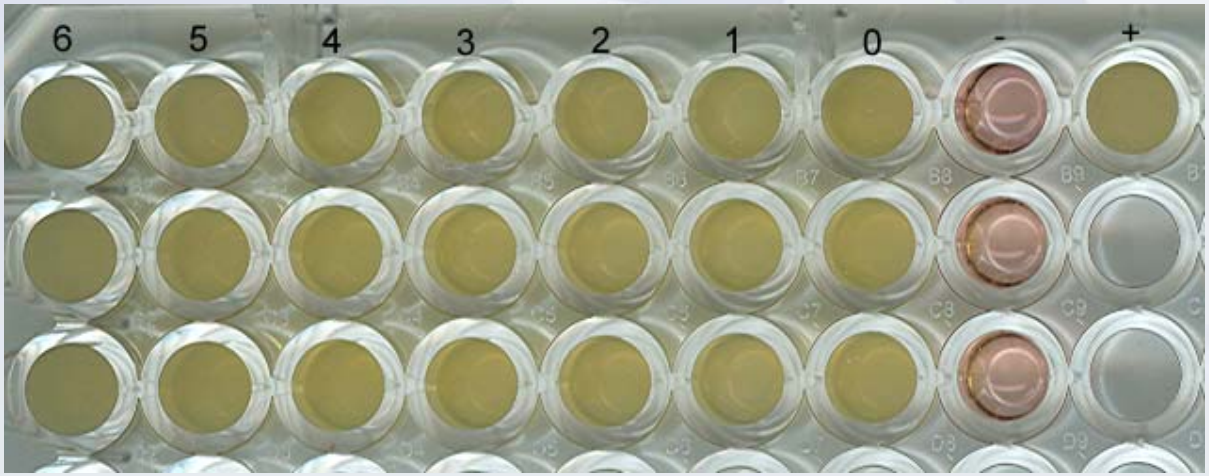


Test 1
Incubated 7 days



Test 2
Incubated 3 days

Testing for Inhibition of Growth



Control

Test

Conclusions

- Biological indicators composed of paper spore strips do absorb formaldehyde during decontamination events
- The concentration of formaldehyde found in the indicators inhibits the growth of spores

Conclusions

- Need to test a non-absorptive carrier such as stainless steel

- Preliminary results show that stainless steel carriers do not absorb formaldehyde

- Using these for BIs does not inhibit the growth of spores