

Evaluation of low-pressure cold plasma for disinfection of ISS grown produce and metal instruments.

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Disinfection using plasma

- Low pressure cold plasma, using breathing air as the plasma gas, has been shown to be effective at precision cleaning aerospace hardware at Kennedy Space Center.
- Both atmospheric and low pressure plasmas are relatively new technologies being investigated for disinfecting agricultural commodities and medical instruments.
- Plasma cleaning is a dry, non-thermal process, which can provide broad-spectrum antimicrobial activity.
- Microgravity compatible since cold plasma uses no liquids and is able to penetrate even the smallest cracks and crevices.

Objectives

- Determine plasma conditions, i.e. vacuum pressure and duration of plasma treatment and any effect on plant tissues.
- Evaluate the efficacy of plasma treatment for produce disinfection.
- Evaluate the efficacy of plasma treatment for disinfection/sterilization of solid items such as utensils and medical supplies.
- Characterize the plasma.

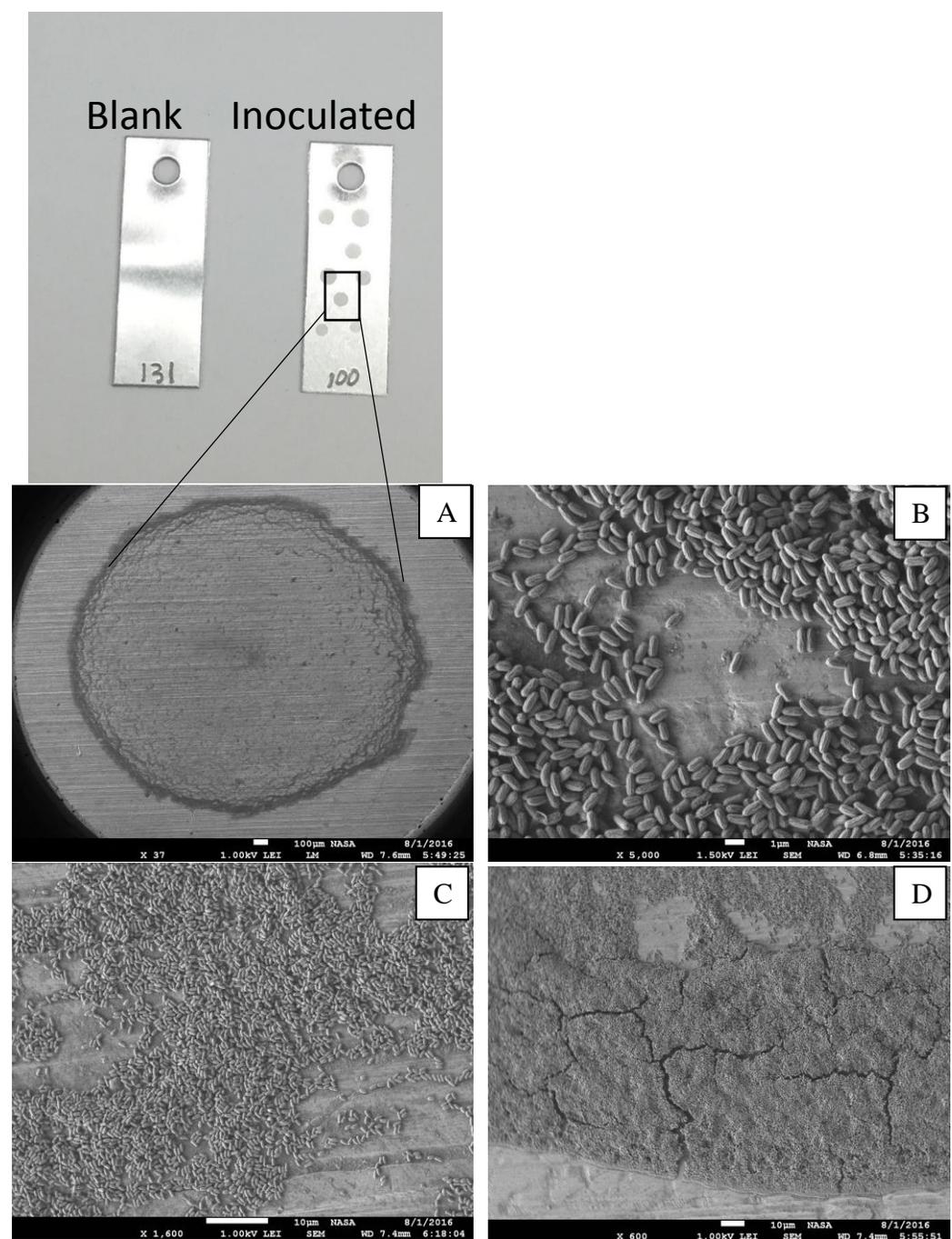
Optimization

- Conditions for metallic coupons were based on previous precision cleaning techniques.
- Moisture present in the produce required adjustments to the low pressure settings to be able to maintain plasma and the integrity of the item for the duration of testing

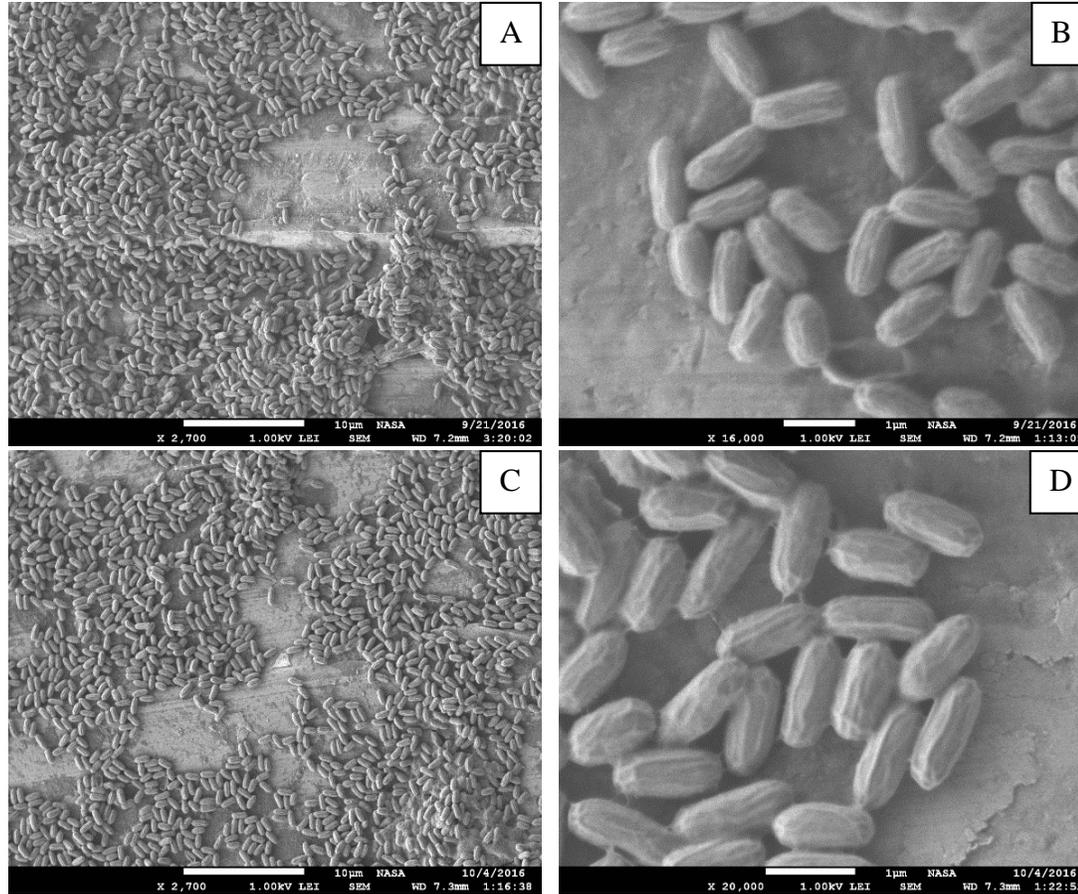
| Item | Pressure (mbar) | Quantity/Run | Exposure Time (min) |
|------------------|-------------------------|--------------|---------------------|
| Metallic Coupons | 0.10 | Up to 10 | 5, 10, 15, 30, 60 |
| Cherry Tomatoes | 0.60 and 0.80 | 5 | 5, 10, 15 |
| Radishes | 0.80 | 3 | 5, 10, 15 |
| Peppers | 0.80 | 2 | 5, 10, 15 |
| Cabbage | Could not be determined | 2 | None |

Metal surfaces

- Coupons were inoculated with $\sim 10^7$ *Bacillus pumilus* SAFR 32 spores per coupon.
- Coupons exposed to plasma at different exposure times, up to 60 min. Controls were subjected to low pressure only.
- Analysis done by:
 - ✓ scanning electron microscopy (SEM) imaging
 - ✓ calculation of log reduction using Most Probable Number technique

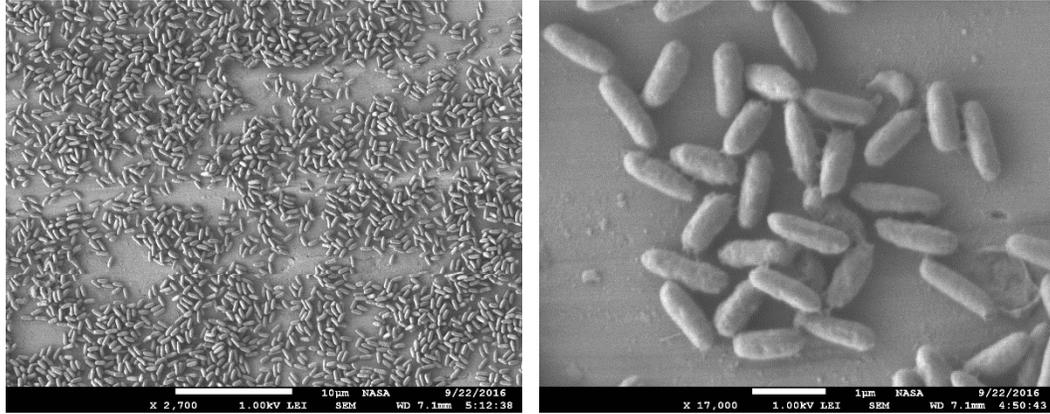


Results (SEM)

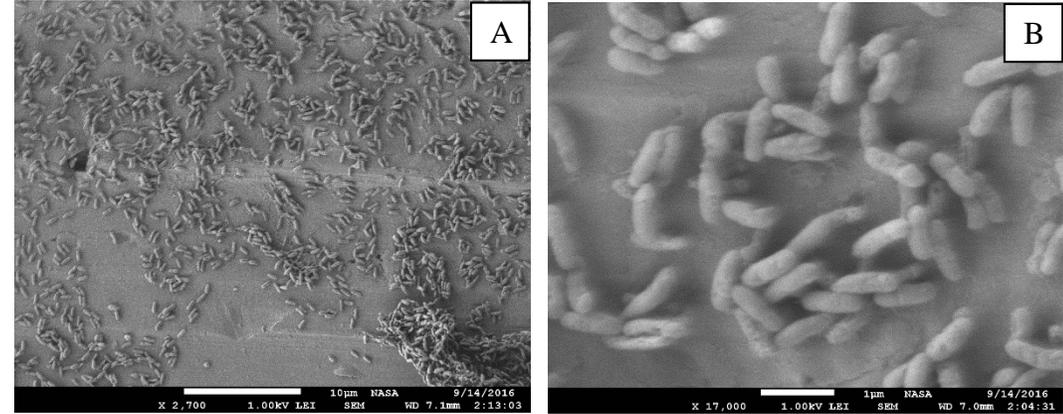


SEM images showing virtually no difference between spores exposed to 15 minutes (A and B) and 60 minutes (C and D) of vacuum.

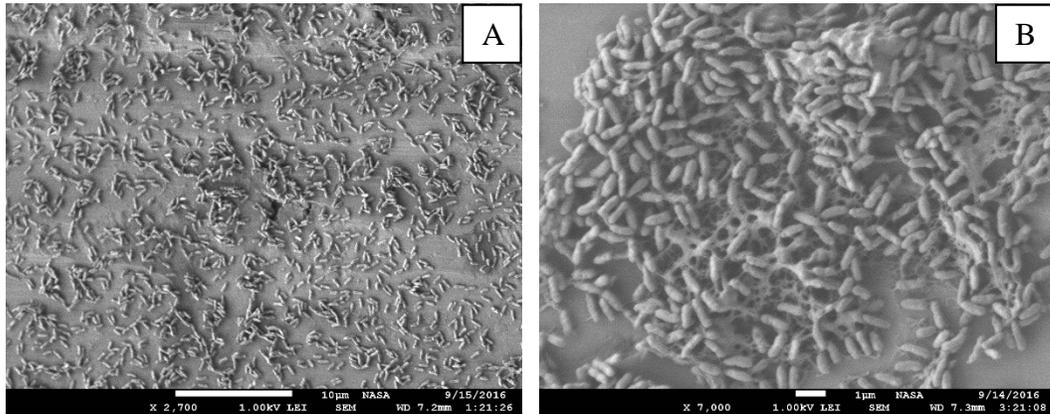
Results (SEM)



SEM images of spores exposed to plasma for 5 minutes.



SEM images of spores exposed to plasma for 15 minutes.

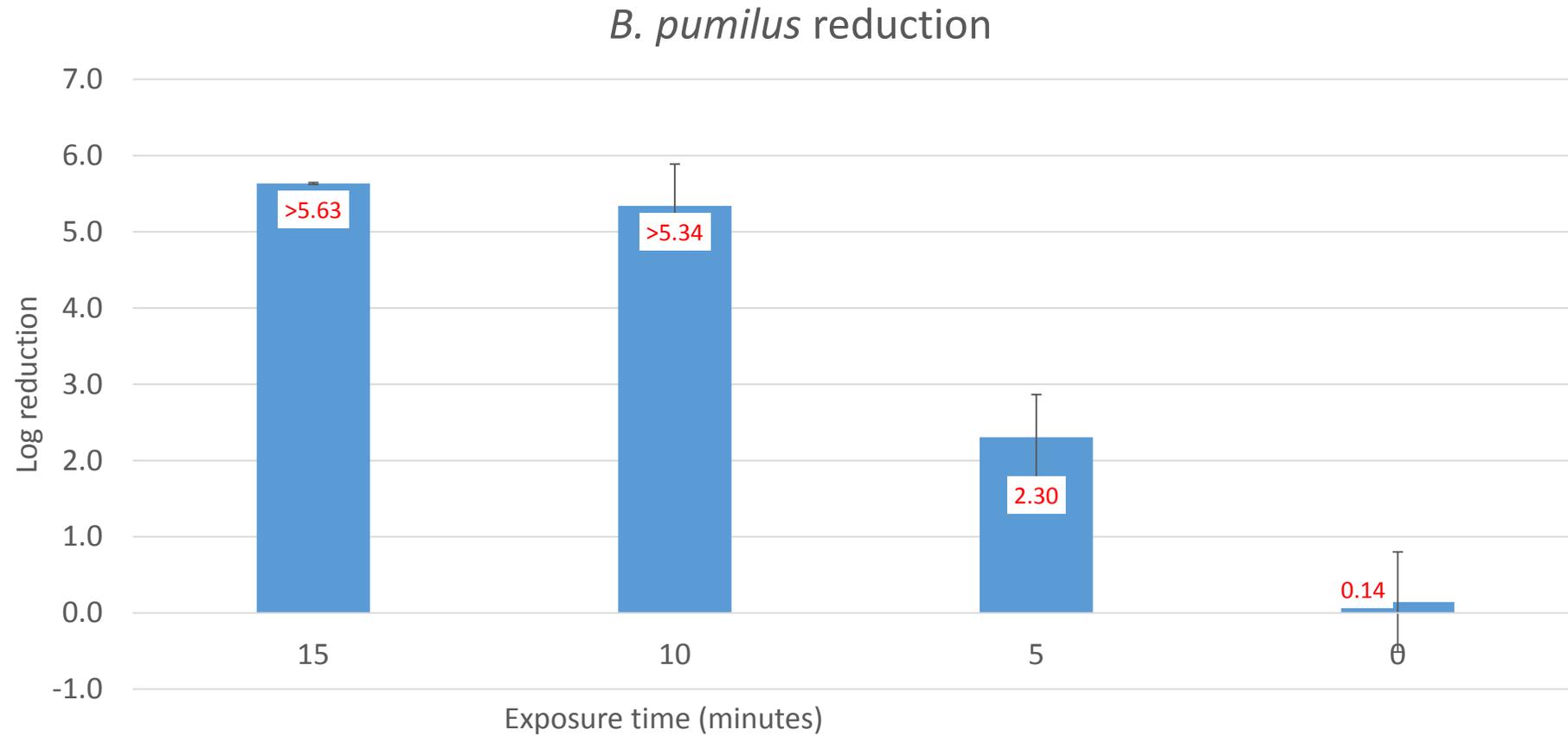


SEM images of spores exposed to plasma for 10 minutes.

Plasma treated spores:

- smaller in size
- Surfaces are smooth and pitted
- Extracellular material evident after 10 and 15 min treatment.

Results. Viability



Produce

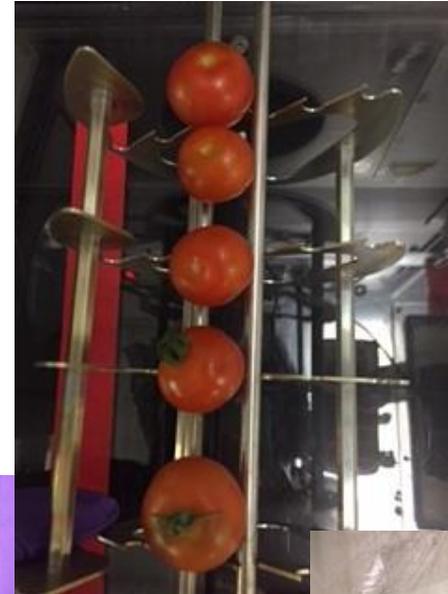
- Produce was grown in controlled environment chambers at KSC.
- Selected candidate crops for Veggie VPU.
- Inoculated with $\sim 10^7$ *Escherichia coli* bacteria/piece.
- Exposure times tested were 0 (low pressure for 15 min), 5, 10 and 15 minutes.
- Analysis done by:
 - ✓ calculation of bacteria log reduction using plate counts
- No changes in temperature were observed when moisture was not present.
- Freezing or tissue damage was detected when water was present.



Inoculated Produce for Plasma Disinfection

- TOMATOES

- ✓ If tomato was very ripe and water detected through removed stalk considerable damage was observed



Inoculated Produce for Plasma Disinfection

- RADISHES

- ✓ Did not present any damage that could lead to test failing



Inoculated Produce for Plasma Disinfection

- PEPPERS

- ✓ Open peppers could lead to test failing and skin damage



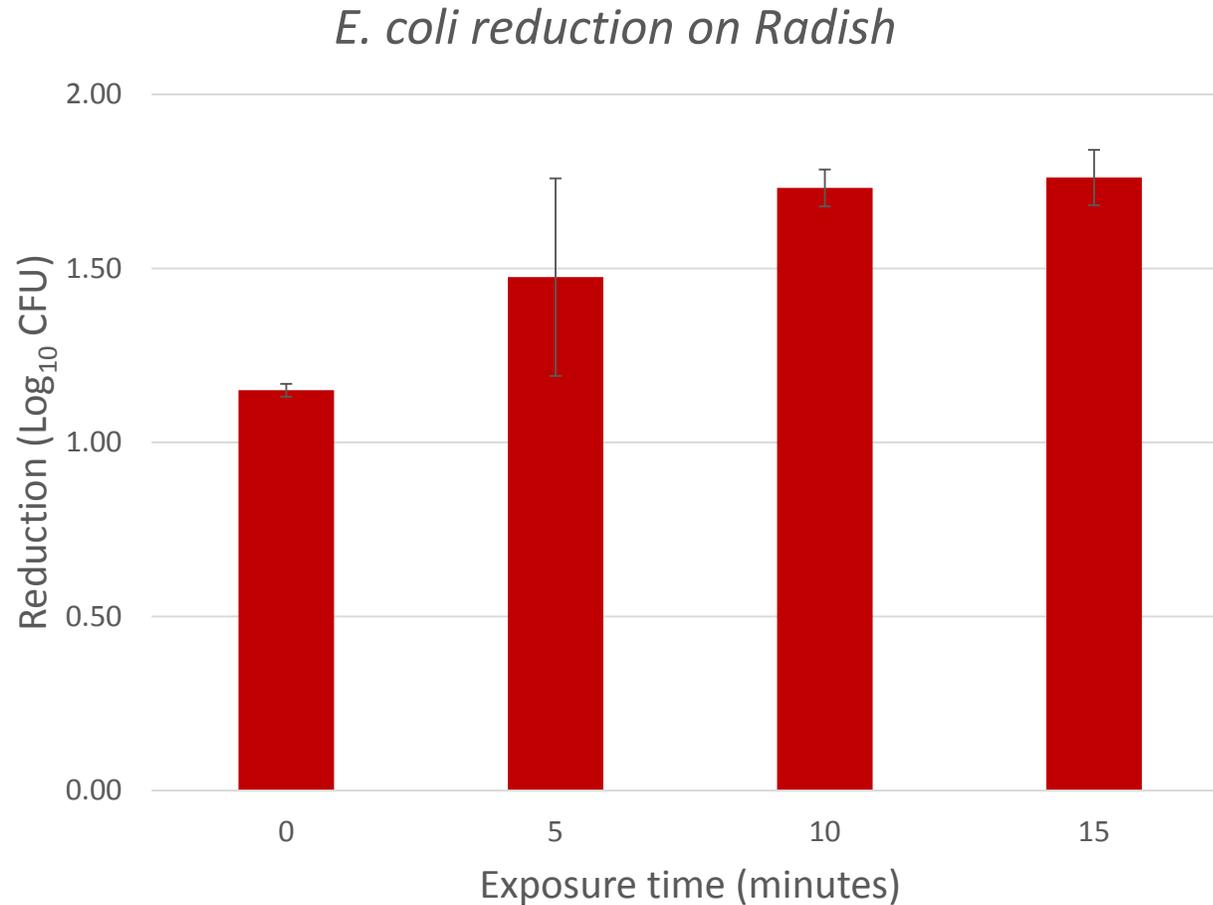
Inoculated Produce for Plasma Disinfection

- CHINESE CABBAGE

- ✓ Failed due to water content
- ✓ Significant tissue damage



Results: produce disinfection



- Average log₁₀ reduction of 1.5, 1.73 and 1.76 for 5, 10 and 15 minute treatments respectively.
- No significant difference between the treatments.
- Reduction was greater than the low pressure control.

Future work

- Test efficacy of process on solid items inoculated with the fungus *Aspergillus niger*.
- Test sterilization of a variety of solid items inoculated with both test organisms.
 - Potable water dispenser needle

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Questions?