Rapid, Quantitative Biological Indicator System with *Bacillus thuringiensis* Al Hakam Spores

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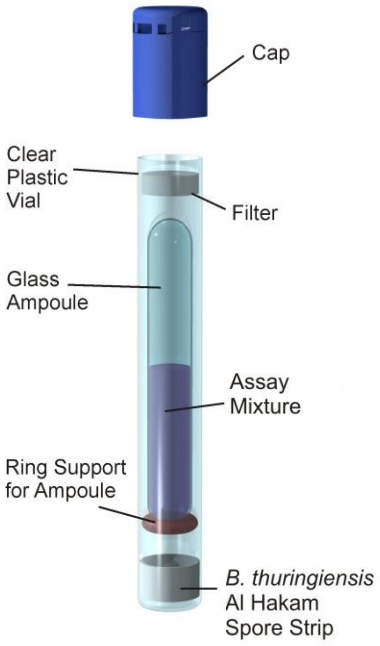
Current Practice of Decontamination Assurance

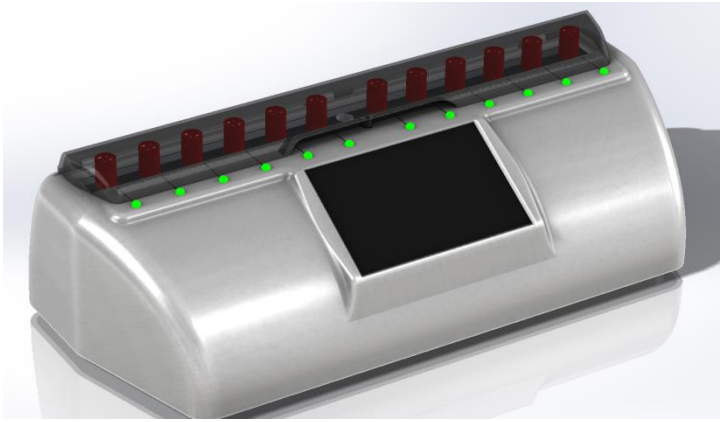
- Surface sampling after decontamination - swabs
- Spore strips or coupons with a known population (biological indicators) placed before decontamination and retrieved afterward

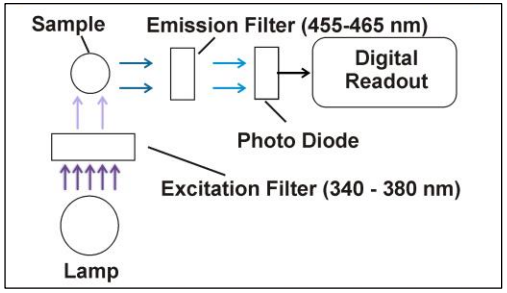
- They are extracted, serially diluted, plated, and enumerated.
- It requires considerable labor.
- Results typically cannot be obtained before 24 – 48 hours, up to 7 days.

Triton's BIs and Detector

- Self-contained BIs and an incubator/detector system
- Quantitative
- *Bacillus thuringiensis* (Bt) Al Hakam
  - A close relative of *B. anthracis,* with the same growth and spore production properties
  - Without pathogenicity: do not have pXO1 or pXO2 plasmids found in *B. anthracis* that encodes for virulence genes and the anti-phagocytic capsule





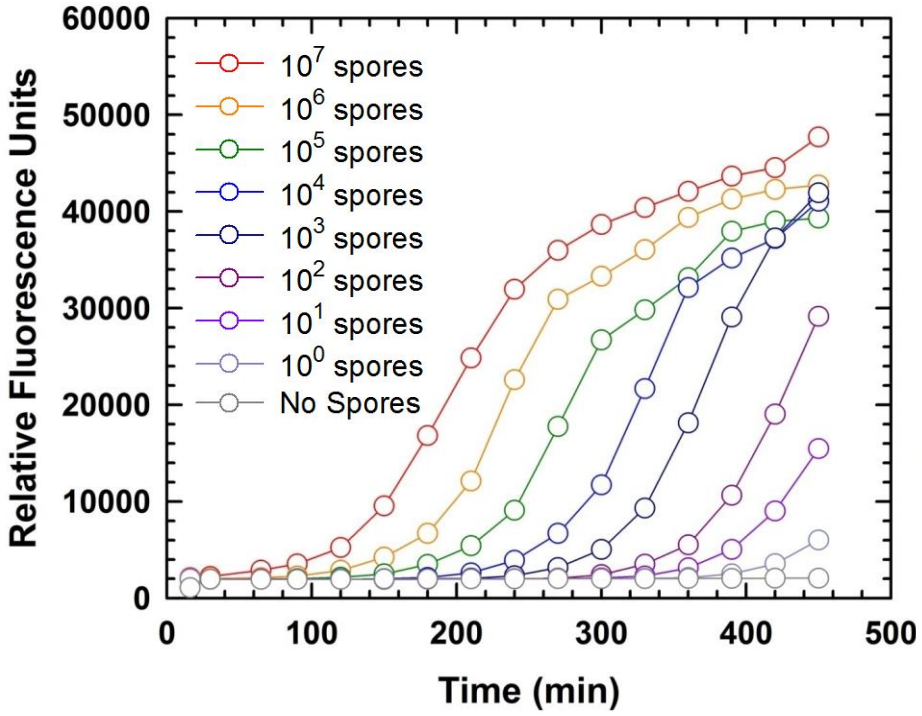


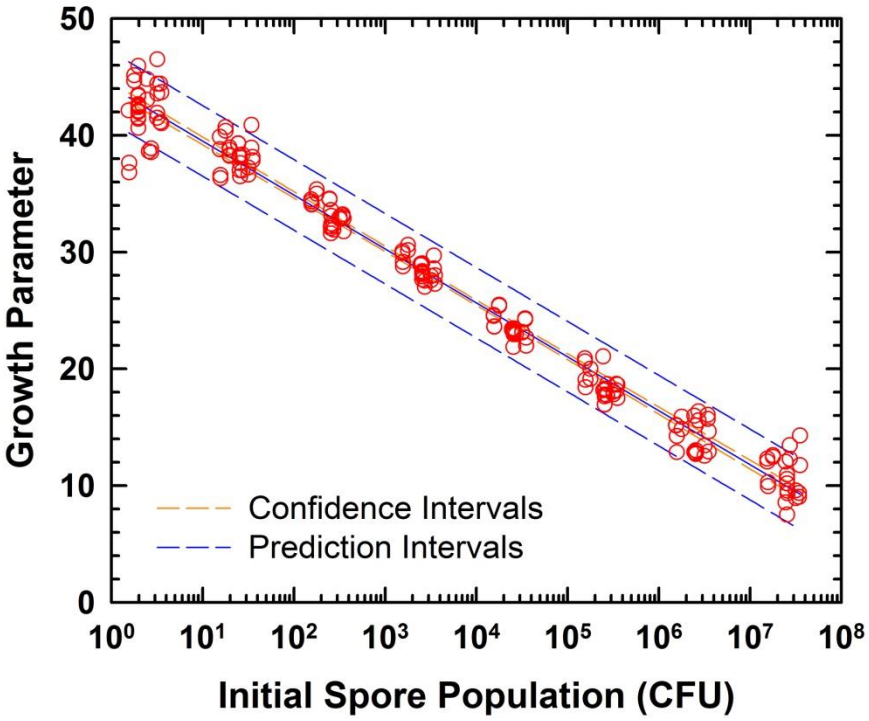
Principle of Assay to Detect Spore Viability

- Evaluates the ability of the spores to germinate and carry out protein synthesis as a measure of the viability of the spores
- Fluorogenic Substrate A
  - Based on the enzyme activity packaged in dormant spores of Bt Al Hakam
  - The enzyme is either not active or not accessible to Substrate A in dormant spores.
  - When the spores germinate, Substrate A is taken up by the spores and hydrolyzed into a highly fluorescent compound by the enzyme.
  - The fluorescence yield is further increased by promoting spore outgrow and vegetative growth.

Quantitative Assay

- 10 different batches were tested.





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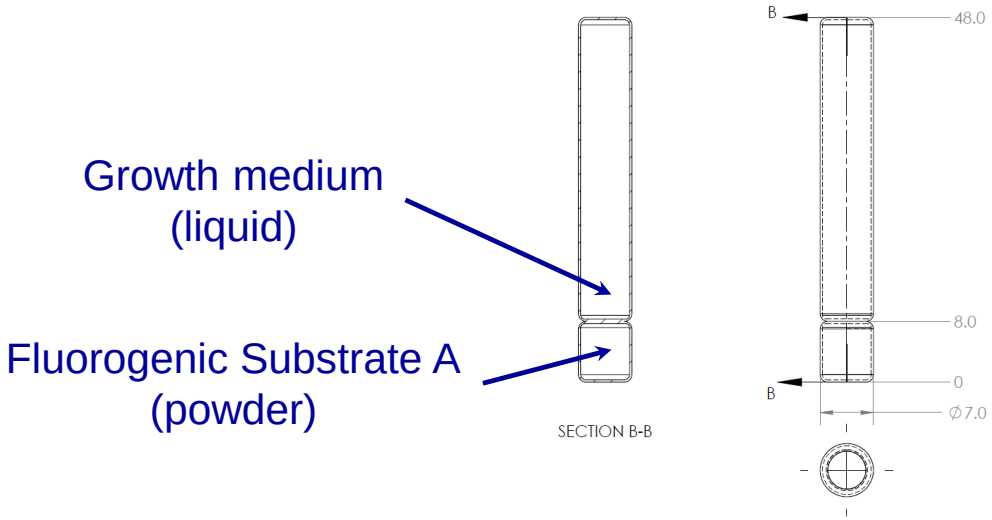
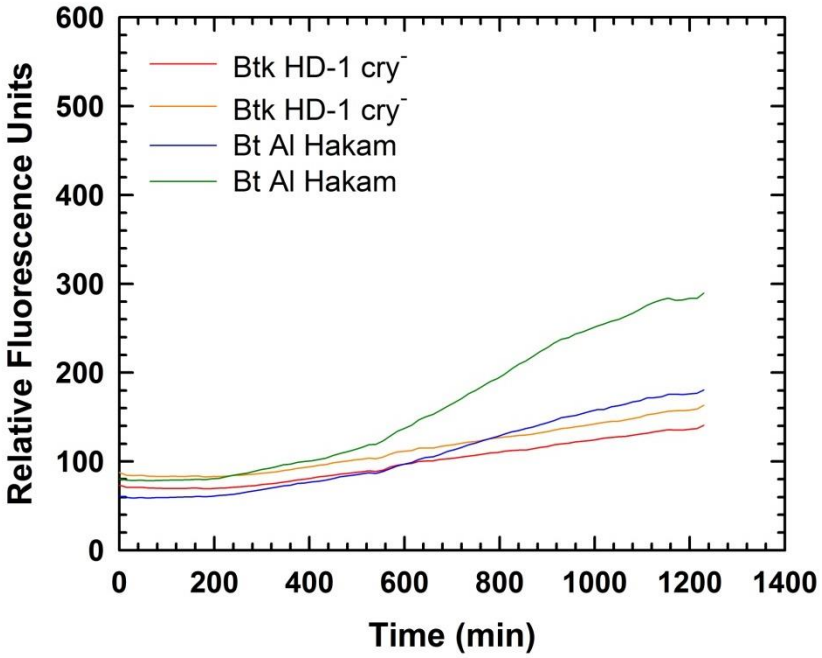
  - The fluorescence yield is further increased by promoting spore outgrow and vegetative growth.
  - Substrate A is heat and moisture sensitive and cannot be autoclaved to sterilize after glass ampoule production.



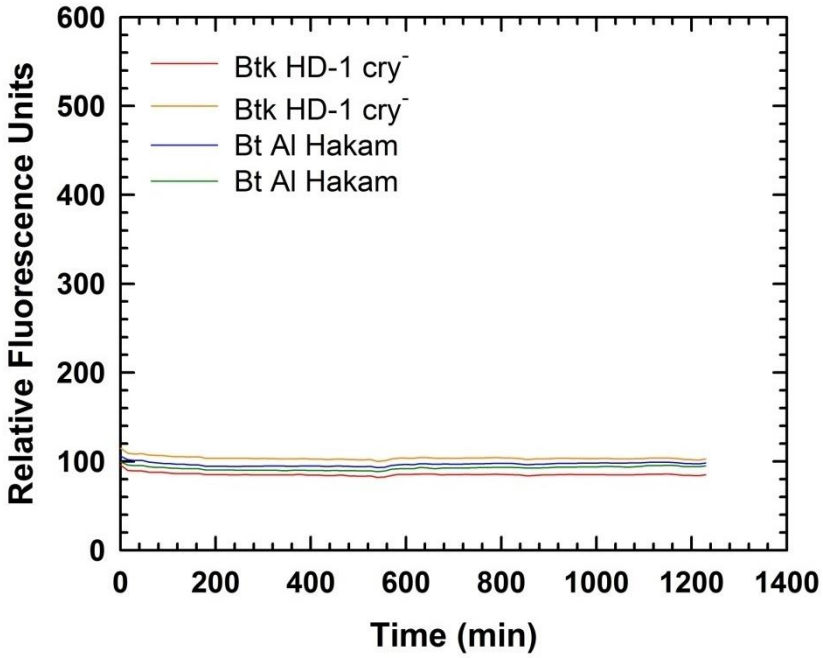
Principle of Assay to Detect Spore Viability

- Fluorogenic Substrate B
  - Based on the enzyme activity synthesized during germination, outgrowth, and vegetative growth
  - Heat and moisture stable – does not require two compartment ampoules

Fluorogenic Substrate B

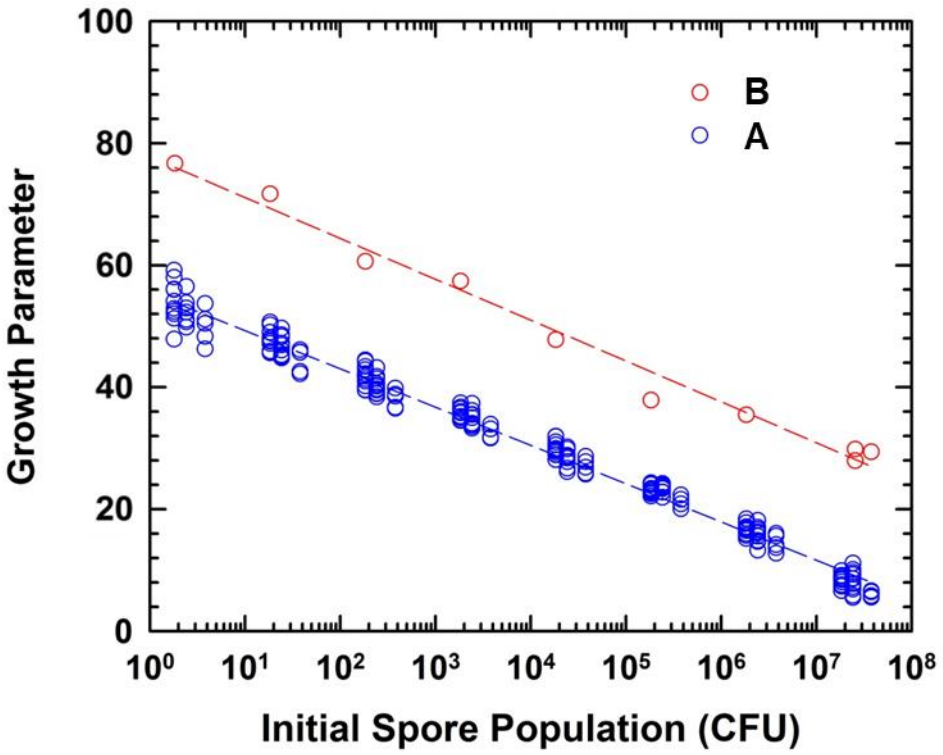


Fluorogenic Substrate C

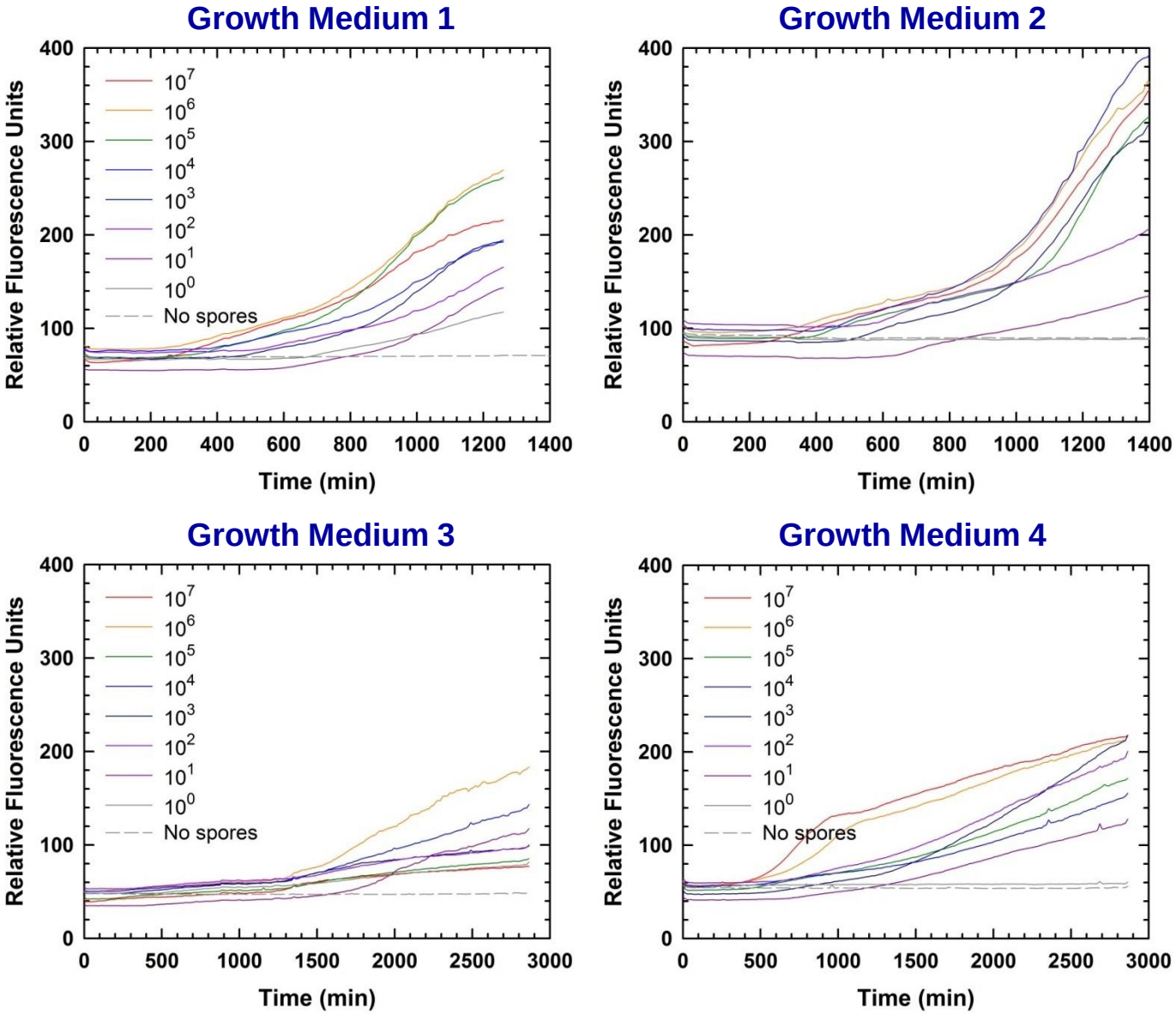


Comparison between Substrates A and B

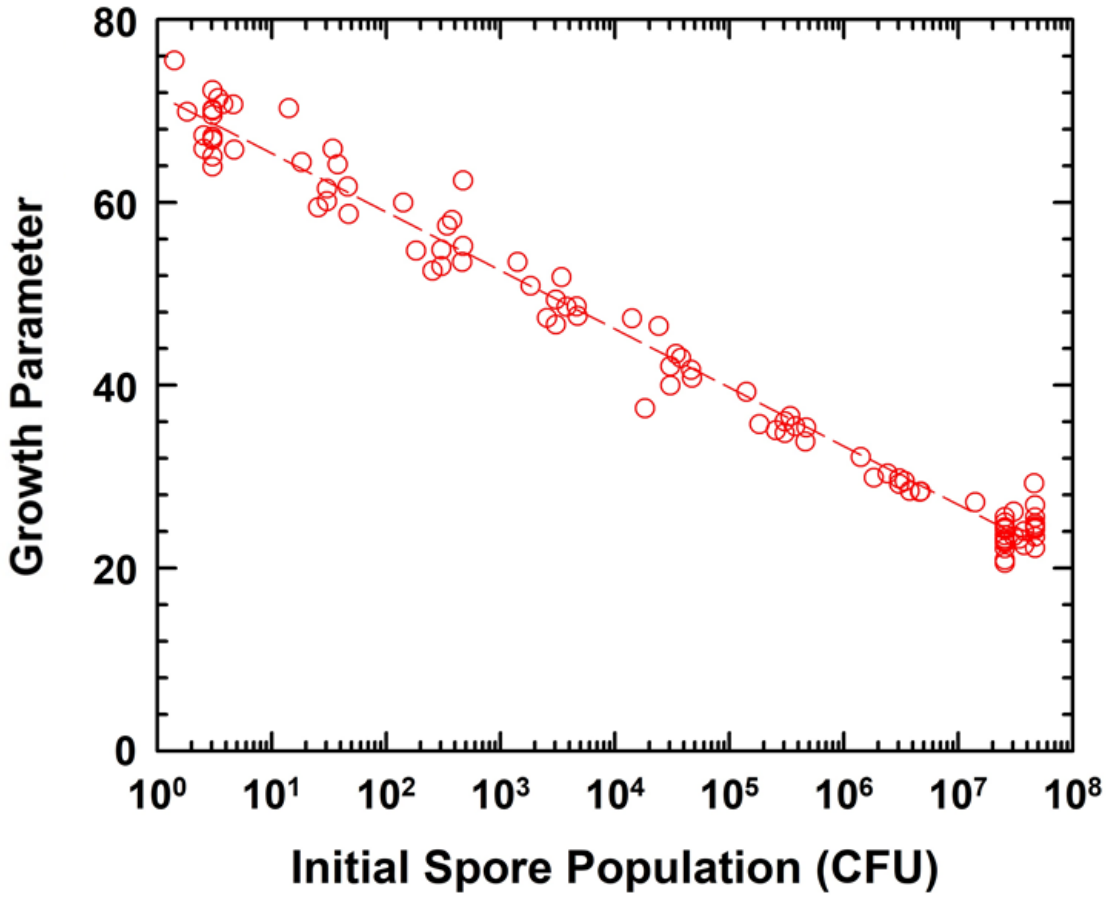
- Both give linear relationships.
- Fluorescence generation is slower with Substrate B.



Growth Medium Optimization for Substrate B

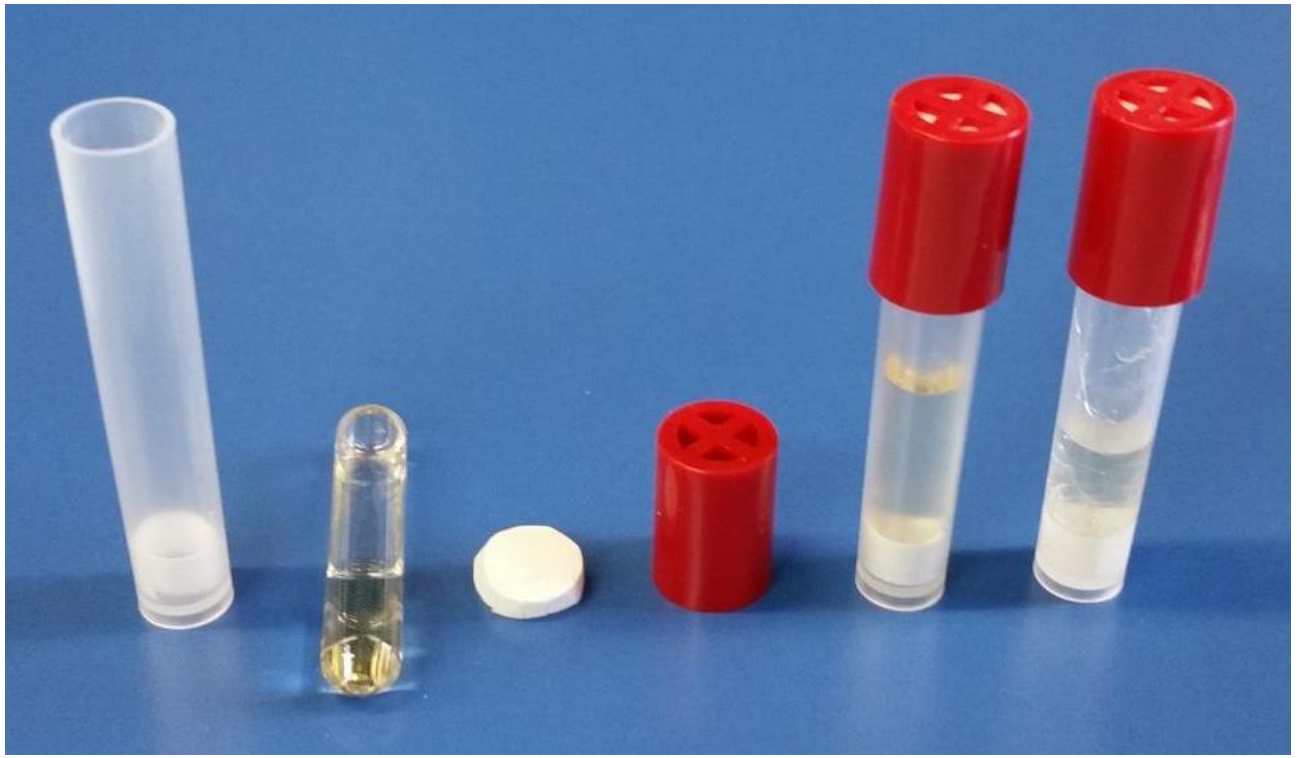


Correlation for Growth Medium 1



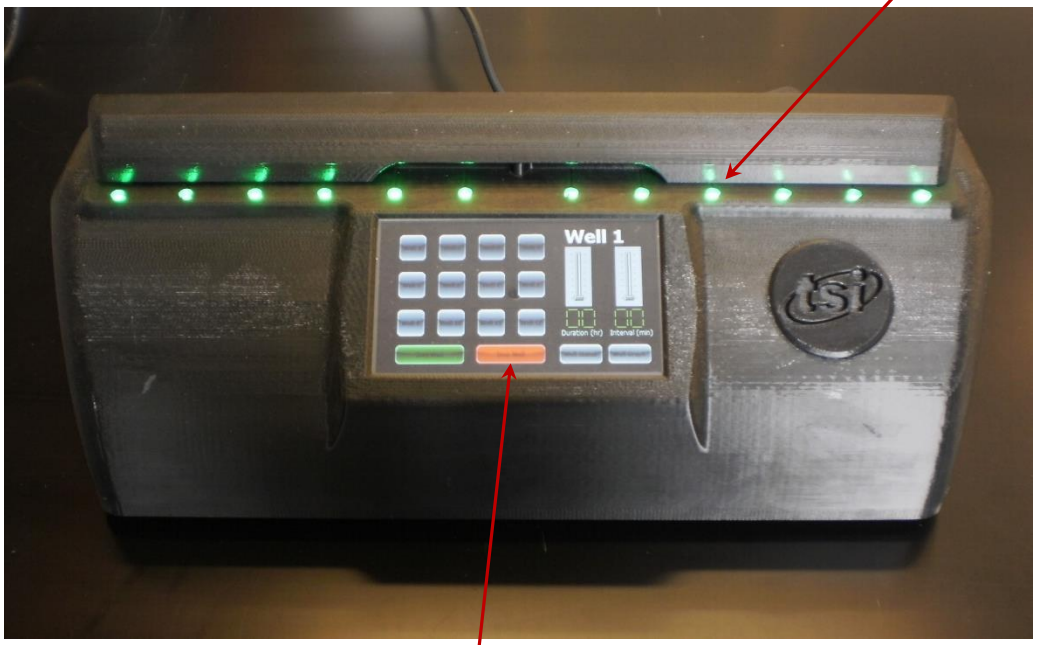
Production of Glass Ampoules

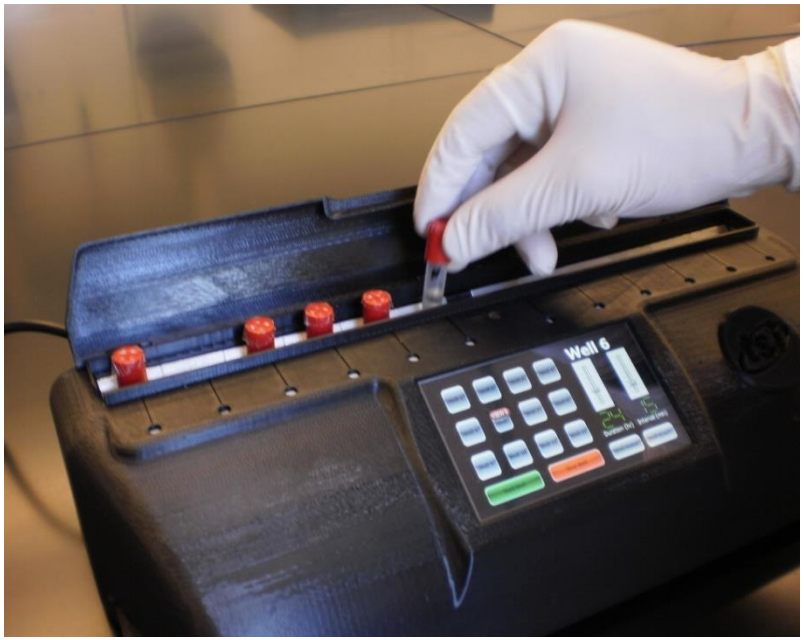
- 7 mm OD × 40 mm H
- Fill volume: 0.6 mL
- 5000 glass ampoules were made and autoclaved to sterilize.
- 6000 more plastic vials and caps were produced.
- Spore strip: inoculated with 10^7 CFU Bt Al Hakam spores



Triton's Detector

- Simultaneous incubation (37°C) and fluorescence detection of 12 BIs
- Touch screen user interface

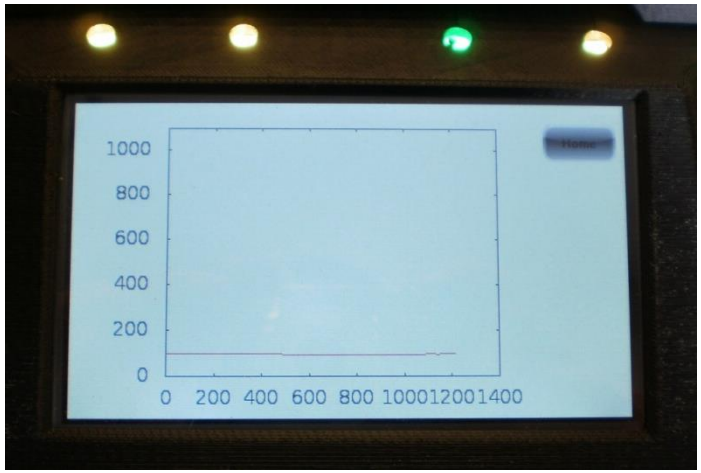


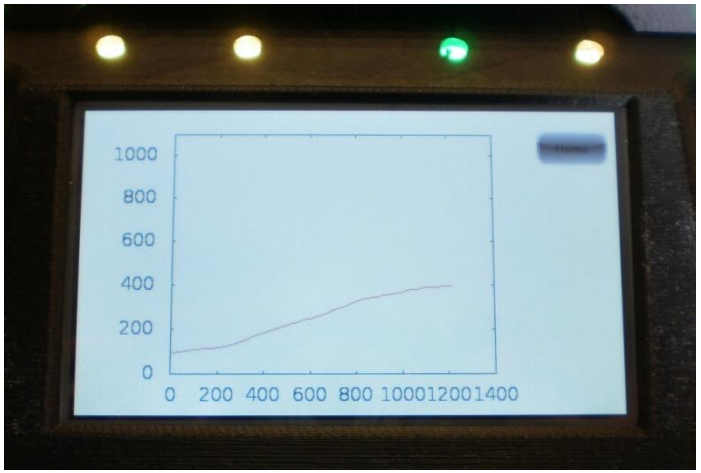


Triton's BIs and Detector

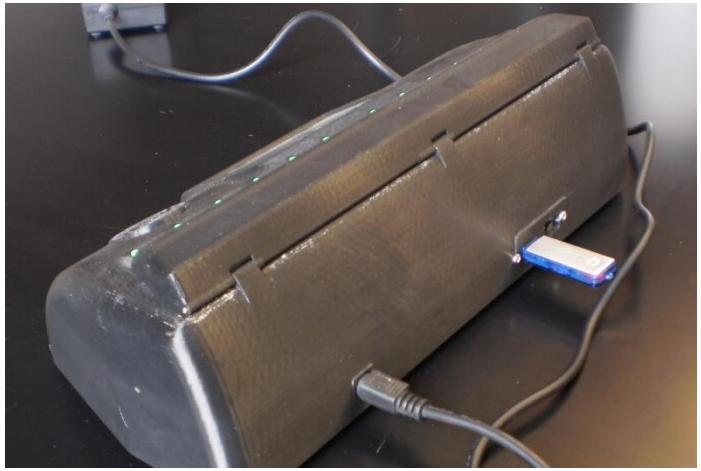
- 10 – 12 hours to detect a single spore → 12 hour run time
- Data processing to display estimated spore population is being implemented.

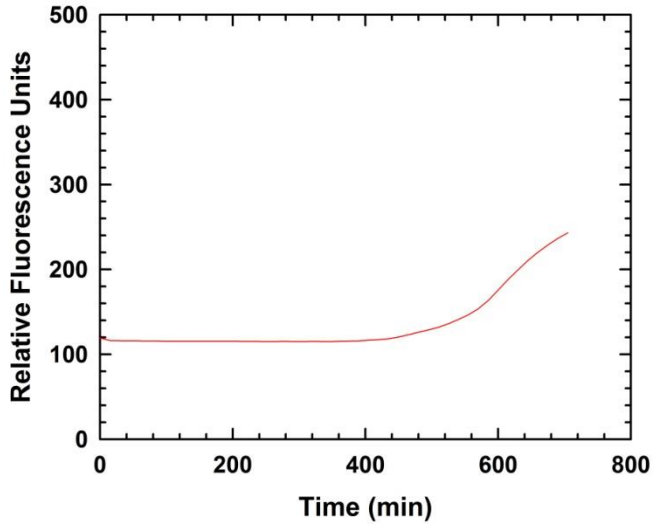
Killed spores





10^7 spores

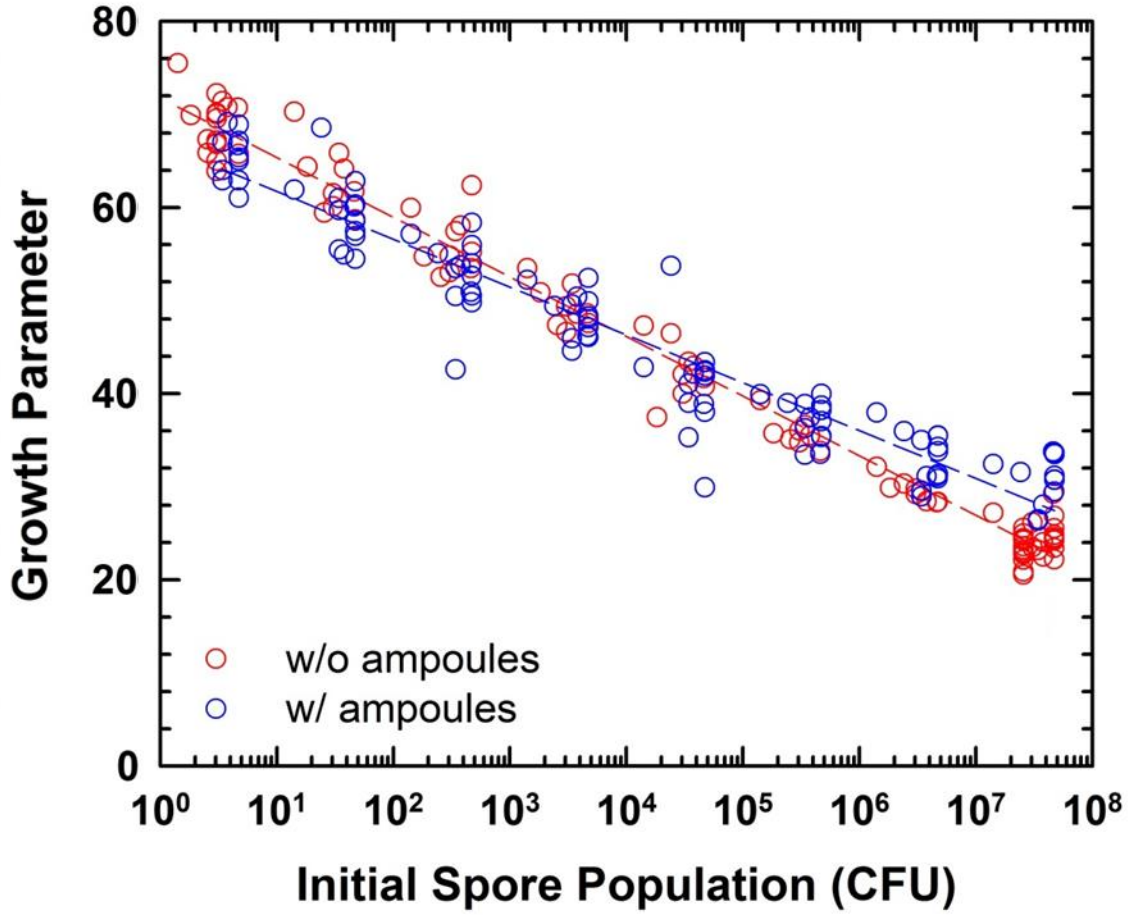




10^0 spores

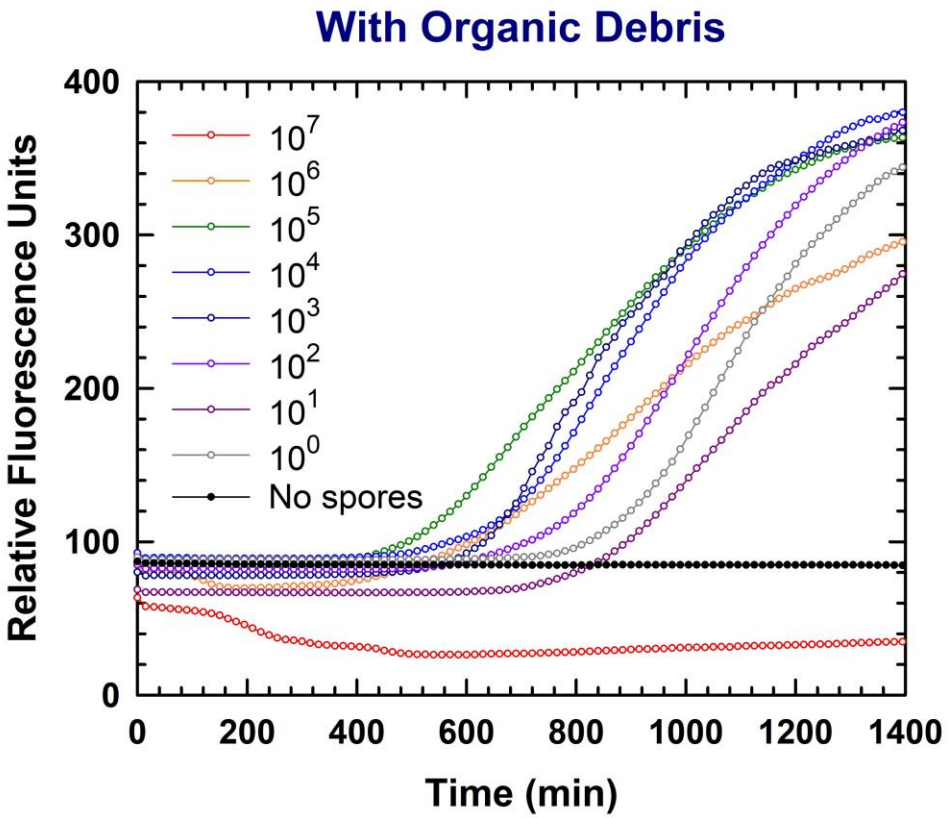
Assay Results with Glass Ampoules

- Glass ampoules cause more scatter in the data.



Spores with Organic Debris

- Spore strip: Bt Al Hakam spores + humic acid
- Buhr et al.[1] – 5 g/L humic acid for 1–2 × 10^8 spores/mL
- 10^7 spores with 250 μg humic acid



[1] *J Appl Microbiol* 2015. 119:1263–1277

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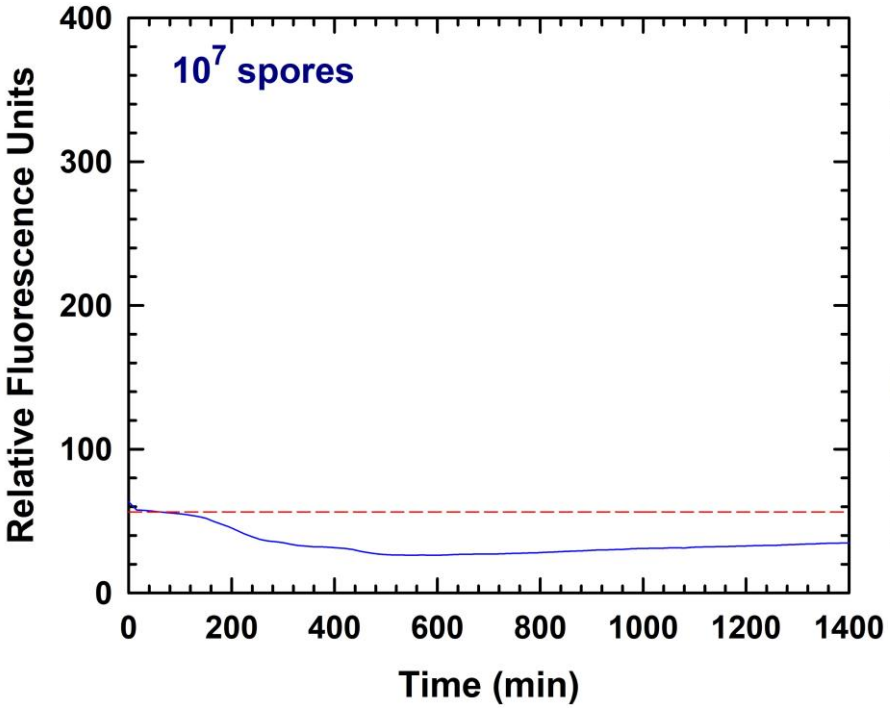

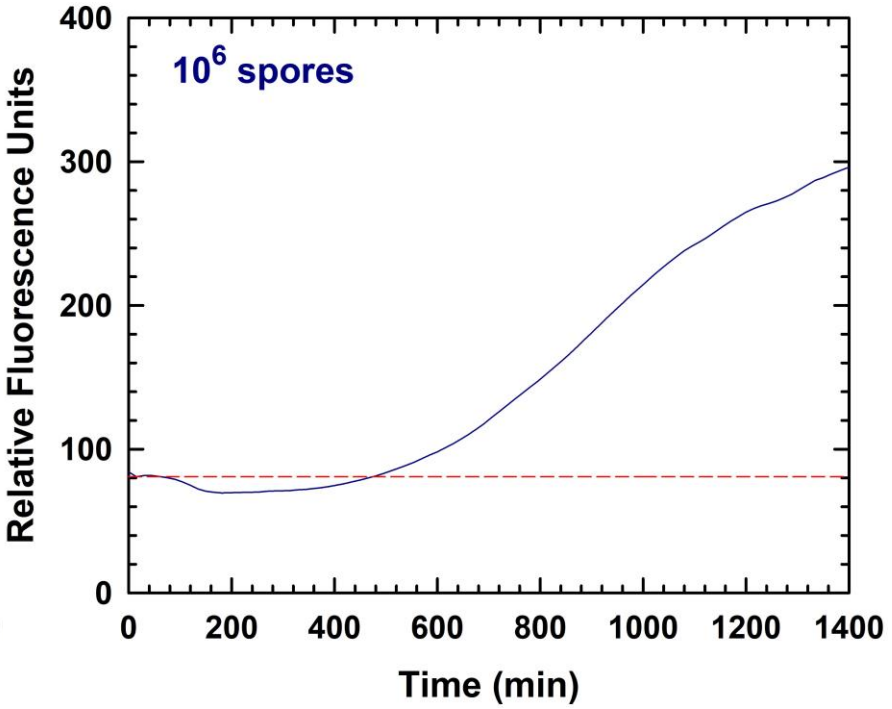

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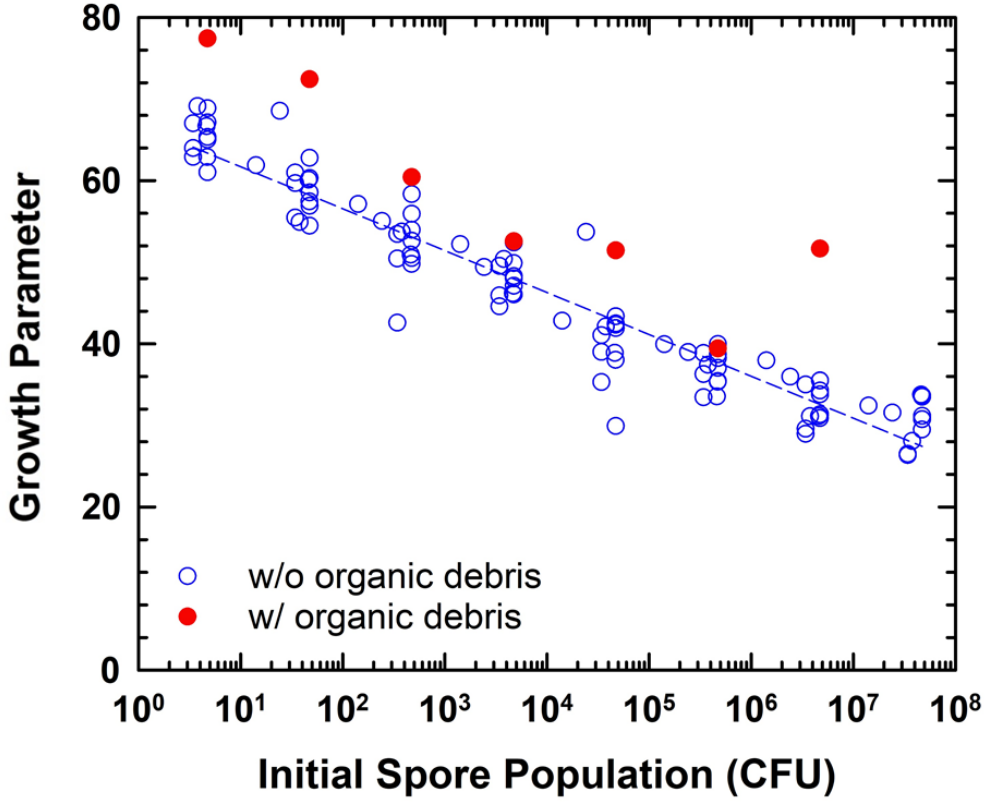

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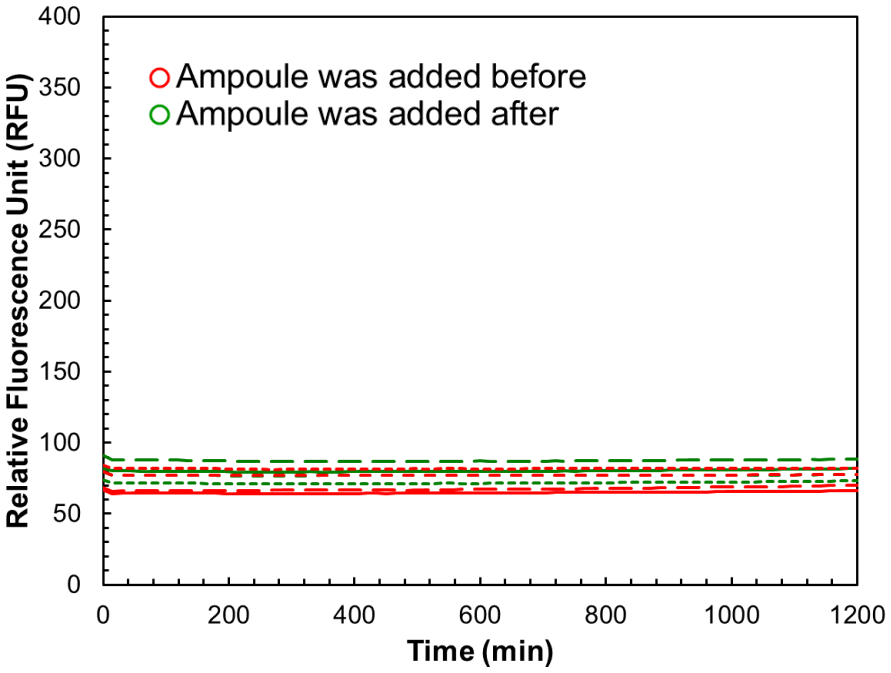
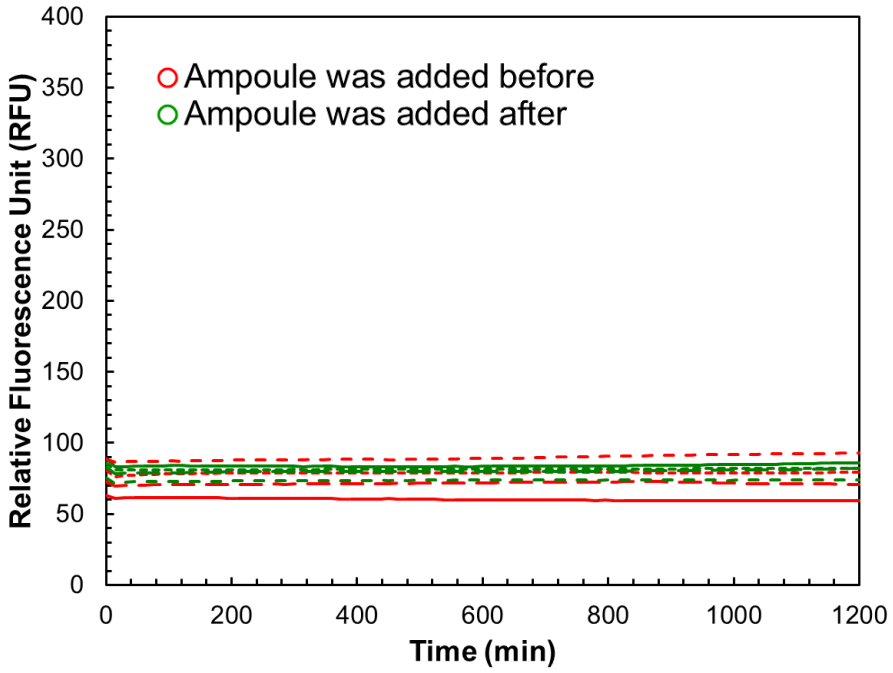
Wet Heat Exposures of BIs

- Tested whether the presence of the ampoule would hinder the action of a sterilant/decontaminant in the BI

121°C, 20 min

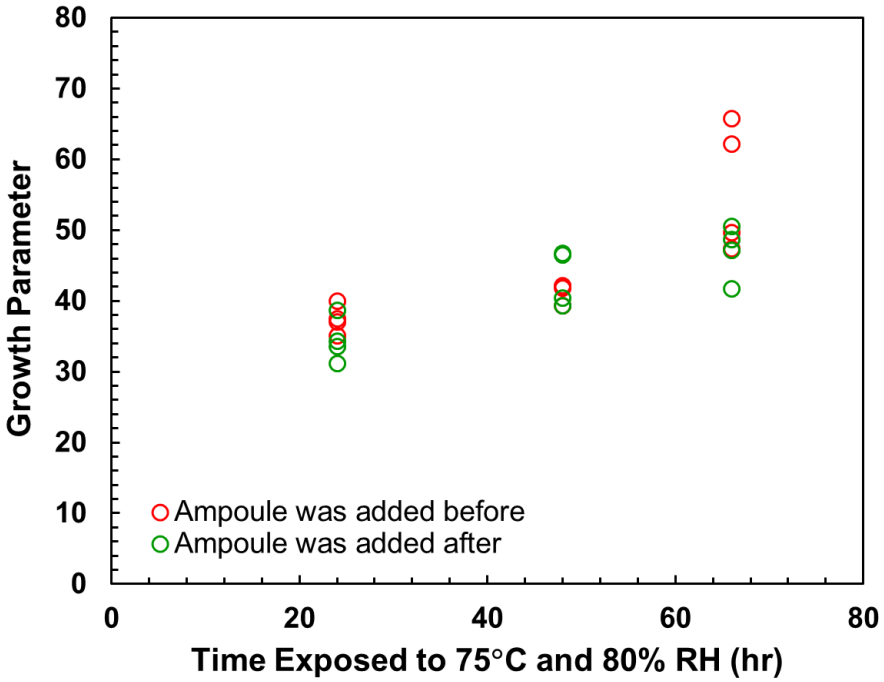


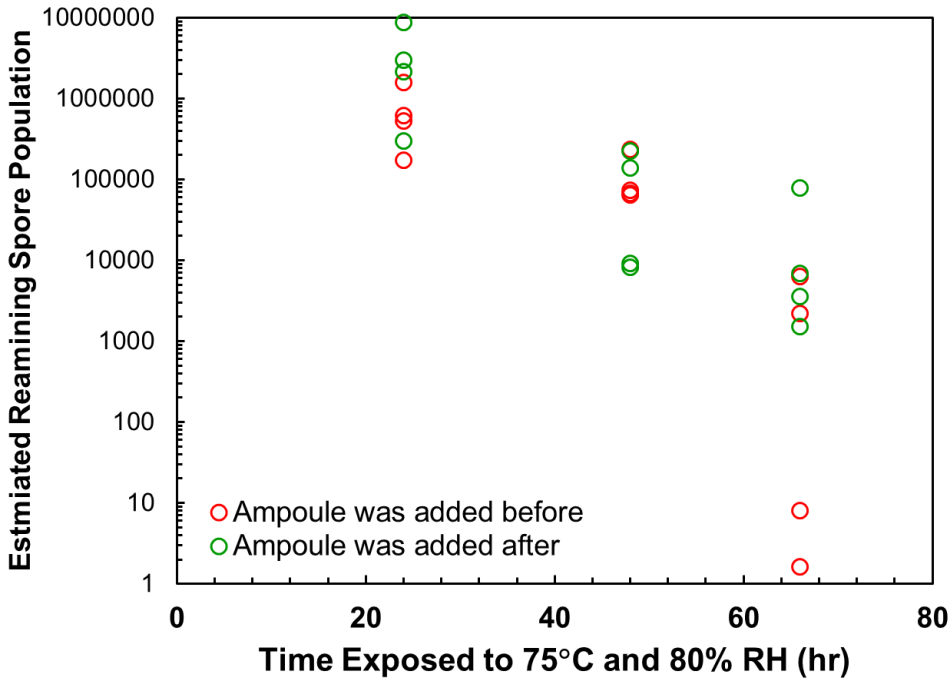
100°C, 10 min



Hot, Humid Air Exposures of BIs

- Tested whether the presence of the ampoule would hinder the action of a sterilant/decontaminant in the Bi
- 75 °C, 80% RH





Summary

- Quantitative biological indicator system with Bt Al Hakam spores
- 10 – 12 hours to detect a single spore and < 3 hours for 10^7 spores
- Allows determination of the decontamination kinetics for modeling to plan decontamination schemes for emergency responses or facilitate developing new decontamination systems for biological agents

- Inclusion of data processing in the detector
- Conversion of the current prototype detector into a manufacturable form with standards compliance

Acknowledgments

- DTRA
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  - Dr. Glenn Lawson
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