ATP BIOLUMINESCENCE-MICROBIOLOGICAL METHODS

FOR THE DETECTION OF CONTAMINATION IN SHORT-LIFE PRODUCTS – USP <73>

Nelson Labs®
A Sotera Health company

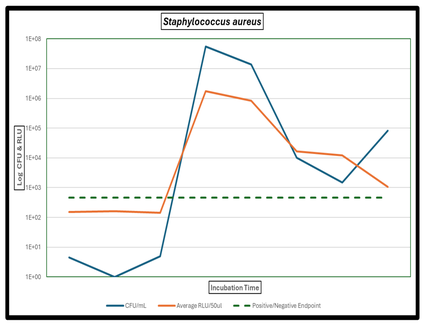
Introduction/Background:

- United States Pharmacopeia (USP) Chapter <73> is a compendial method for Short-Life Products.
- USP <73> released in Aug. 2025
- Adenosine triphosphate (ATP) bioluminescence is a recommended technology for release of short-life products.
- The assay uses a luciferase enzyme as a catalyst. Any ATP present reacts and produces a photon of light.
- When microbial contamination is present the elevated levels of light will be measured on a luminometer.

Test Setup:

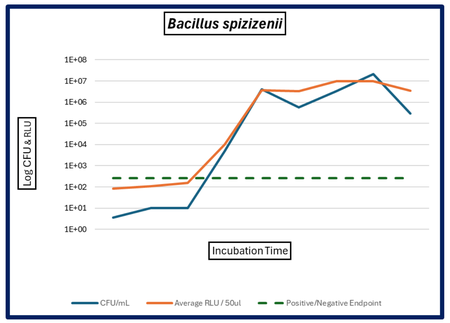
- Aseptically inoculated nutrient growth media with <10 CFU.
- Incubated the inoculated media at 20-25° C and 30-35° C (aerobic & anaerobic microbes).
- Removed aliquots at various time points to perform plate counts and bioluminescence testing.
- Performed rapid sterility detection on the Celsis Advance II instrument.
- Utilized Relative Light Unit (RLU) data to help determine plate count dilutions to perform.

Test Results:



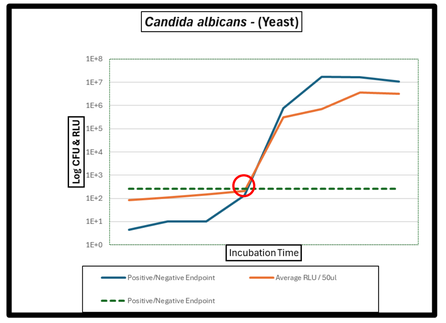
Observations:

- The Relative Light Units (RLU) for the bioluminescence testing – (orange line) initially leads the microbial count (blue line) for this Gram-positive microorganism. Amplification reagents used in the rapid testing may be increasing ATP for quicker detection.
- Both start to show exponential growth at approximately 15h in the line chart above.
- Both reach thresholds at similar times.



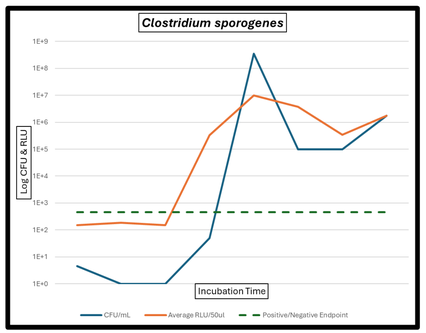
Observations:

- The Relative Light Units (RLU) for the bioluminescence testing – (orange line) initially leads the microbial count (blue line) for this Gram-positive microorganism. Amplification reagents used in the rapid testing may be increasing ATP for quicker detection.
- Both start to show exponential growth between 11-24h in the line chart above.
- Both reach thresholds at similar times.



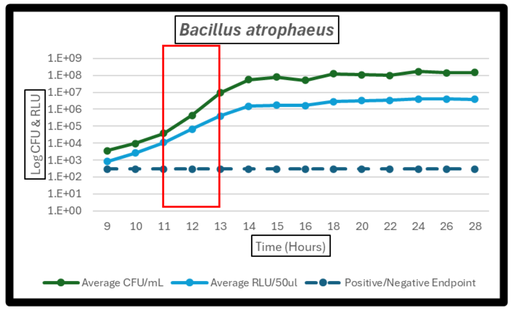
Observations:

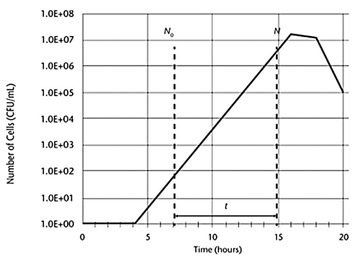
- Fungal-Yeast incubation time is typically longer than bacterial growth for RLU & visual detection.
- Both start to show exponential growth between 27h – 51h as observed circled in red in the line chart above.
- Both reflect similar thresholds at similar times.
- High speed vortexing/mixing is often necessary for filamentous mold to release ATP from fungal hyphae.

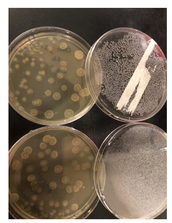


Observations:

Anaerobic microbial detection using ATP-bioluminescence appears to occur between 11-27h of incubation. This was quicker than visual bacterial growth on agar plates. The exponential growth started earlier with the Rapid RLU method when compared to microbial colony counts for above testing. The amplification process for rapid testing may produce earlier detection







Calculation of Generation Time for *Bacillus atrophaeus*

Bacillus atrophaeus	Incubation Time (h)	Dilution (10^x)	Volume Plated (mL)	Plate 1 (CFU)	Plate 2 (CFU)	Average (CFU)	CFU/mL	Generation Time (h)
Beginning	9	-1	0.1	29	28	28.5	2.85E+03	1.01
End	10	-1	0.1	59	55	57	5.70E+03	
Beginning	10	-1	0.1	59	55	57	5.70E+03	0.47
End	11	-2	0.1	25	26	25.5	2.55E+04	
Beginning	11	-2	0.1	25	26	25.5	2.55E+04	0.33
End	12	-3	0.1	15	27	21	2.10E+05	
Beginning	12	-3	0.1	15	27	21	2.10E+05	0.25
End	13	-4	0.1	33	33	33	3.30E+06	
Beginning	13	-4	0.1	33	33	33	3.30E+06	0.29
End	14	-5	0.1	37	37	37	3.70E+07	
Beginning	14	-5	0.1	37	37	37	3.70E+07	1.61
End	15	-5	0.1	54	60	57	5.70E+07	
Beginning	11	-2	0.1	25	26	25.5	2.55E+04	0.29
End	13	-4	0.1	33	33	33	3.30E+06	

Calculation of Generation Time (time required for microbial cell number to double):

$G = t / 3.3 \times \log_{10} (N / N_0)$

where:

T = time interval (h) – (interval measured in exponential phase)
N = number of cells/CFU at the end of the time interval
N_0 = number of cells/CFU at beginning of the time interval

Determination of Product Incubation Time

The incubation time for product microbial detection is calculated as:

$T = t_{ttd} + \log_2 (10) \times G$

where:

T = incubation time for microbial detection in the product to be examined (h)
t_{ttd} = longest time to detection in the method suitability test (h)
G = generation time slowest growing microorganism (h)

Conclusions:

- USP bacterial microorganisms were detected with rapid ATP-Bioluminescence typically from 11-48 hours incubation
- USP fungal microorganisms were detected with rapid ATP-Bioluminescence typically from 24-70 hours incubation.
- ATP-Bioluminescence Relative Light Unit (RLU) results typically mimic same patterns as CFU plate counts data.
- Testing & understanding of product bioburden with calculation of generation time(s) are required to determine shorted sterility incubation times for short-life products using this method.

References & Acknowledgements:

(1) USP <73> ATP BIOLUMINESCENCE-BASED MICROBIAL METHODS FOR THE DETECTION OF CONTAMINATION IN SHORT-LIFE PRODUCTS.
(2) Nelson Laboratories, LLC personnel - Design Qualification (DQ) *Scott Dimond – Testing, Data & Results, *Daniel Olsen - Protocol, Science, Statistical & Validation Support. *Amina Rahmoune Lab Operation Manager (in photo)
(2) Celsis Advance II Instrument reagents, literature & supplies.