# Evaluation of ATP-bioluminescence detection as a supportive technology for bioburden and sterility testing processes.

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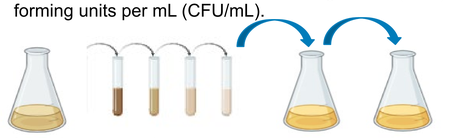
## Introduction

**Sterility and bioburden testing is used by biopharmaceutical manufacturers to ensure product quality and patient safety, as well as to meet regulatory requirements. Sterility testing is the examination of the product for macroscopic evidence of microbial growth whereas bioburden testing is the quantification of microbial load. Biologic products and other sterile drugs made using aseptic processes are expected to report "no growth" and typically a bioburden result greater than 100 CFU/10 mL. Compendial methods such as enumeration by surface-spread plating (USP<61>) for bioburden and direct inoculation (USP<71>) for sterility are common analytical procedures, however, test results can take between 3-7 days and 14 days, respectively. Pharmaceutical manufacturing processes may be put on hold and finished drug product may not be released until sterility/bioburden results are available. Compendial methods for in-process control testing may not fully support evolving trends in biologic products such as Continuous Manufacturing, Process Analytical Technology for downstream operations, and Real-Time Release Testing that require quick process feedback. Development of rapid/alternative testing methods with comparable detection performance but reduced time to result could offer significant benefits to the pharmaceutical manufacturing industry.**

**Rapid Microbial Methods (RMMs) are alternative assays or technology platforms to perform sterility or bioburden testing that may release critical data at a faster rate than the compendial methods. However, these commercially available methods are not widely adopted due to potential performance and regulatory challenges for implementation. ATP amplification detection technology is a proposed RMM for bioburden and sterility testing with results available at approximately 18-24hrs and 5 days, respectively. The following research evaluates the performance of this technology to assess detection of microbial contamination with an accelerated enrichment period.**

## Materials and Methods

Bacteria cultures were prepared using cryopreserved strains of USP and/or biotechnology product relevant organisms. All species are tested for growth promotion with tryptic soy broth (TSB) and tryptic soy agar (TSA). Celsis Accel assay performs ATP amplification within viable organisms while in liquid suspension. The background luminescence of sample matrix (TSB) establishes positive detection threshold using system recommended default setting of 2x for sterility and 3x for bioburden assays. Results were given in relative light units (RLU). Enumeration of surface-spread plating is calculated as colony forming units per mL (CFU/mL).

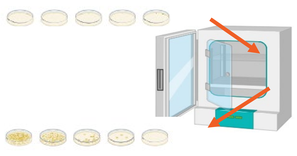


**Sample Preparation**

**Reduced Enrichment Period**

**Celsis Accel Assay**

**Visual inspection**



**Enumeration**

**Figure 1.** Materials Overview (*image created with BioRender.com*)

| Process | LOD/Bioburden | Sterility |
|---|---|---|
| Sample Preparation (TSB) | Test articles >0.01 CFU/mL | Test articles ≤10 CFU/mL and ≤0.01 CFU/mL |
| Reduced Enrichment Period | Shaking incubator at 130rpm (35°C), sampled at 0hr, 4hr, and 24hr | |
| Celsis Accel & AMPiScreen kit (Charles River Laboratories) | 50ul processed in duplicate at 0, 4, and 24hr<br>Day 6 run for Sterility samples if needed | |
| Enumeration (TSA) | 0.1mL plated in duplicate, read next day | |
| Visual Inspection | N/A | Visual reporting of turbidity performed up to Day 6 |

**Table 1.** Methods overview for bioburden and sterility testing.

## Results and Discussion

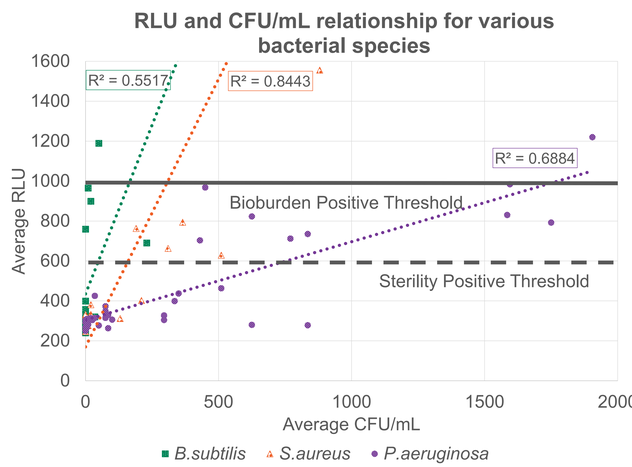



**Figure 2.** Representative graph showing microbial load detection using Celsis system with Sterility threshold (dashed line) and Bioburden threshold (solid line).

| *B. subtilis* | | | | |
|---|---|---|---|---|
| **Enrichment** | **CFU/mL** | **Celsis RLU** | **Celsis Result (Bioburden)** | **Celsis Result (Sterility*)** |
| 0hr | 5 | 532 | Neg | Pos |
| 4hr | 485 | 674 | Pos | Pos |
| 24hr | Lawn | 99999999 | Overload | Overload |

**Table 2.** Example dataset for a given test article <10CFU/ml enriched over time illustrating instrument read out and signal differences to enumeration method. *Reanalysis of data with Sterility settings.

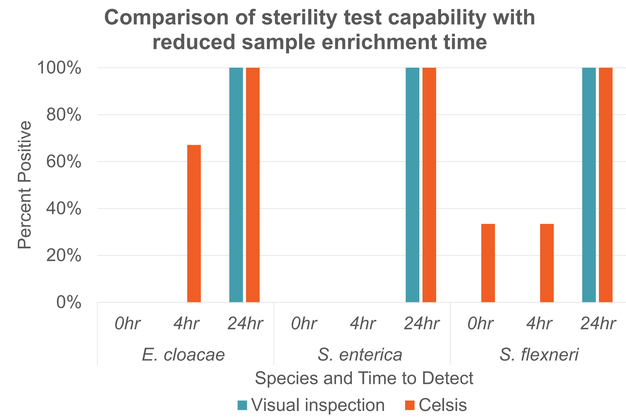



**Figure 3.** Representative graph of Sterility detection rates for samples with simulated occult contamination levels after various enrichment periods (n=3).

**Quantification of microbial load**

- **Figure 2** demonstrates RLU measurement does not directly indicate microbial contamination level
- The $R^2$ values seen in **Figure 2** indicates a weak relationship between RLU and CFU/mL
- ATP content/amplification varies, likely due to number of metabolic pathways available to different bacterial species

**Enrichment time**

- **Table 2** suggests that reduced enrichment yields enumerable results for bioburden analysis, however, ATP-detection fails to detect even substantial contamination
- Reanalysis with sterility setting for ATP-detection suggests increased detectability with lowered threshold

**Detection of occult contamination**

- **Figure 3** Demonstrates that 24hr sample enrichment generates similar performance results to visual inspection when challenged with a concentration of ≤10 CFU/mL
- Detection earlier than 24hr may be possible but inconsistent
- Test articles with a concentration of ≤0.01 CFU/mL were negative for both ATP-detection and visual inspection for all enrichment periods (data not shown)

## Conclusion

**The aim of this research is to evaluate performance of ATP amplification in liquid suspension detection technology as a rapid/alternative microbial method. This investigation includes a reduced enrichment period which may be a supportive application for biopharmaceutical industry trends requiring quicker time to result for microbial detection. Our findings indicate that:**

- **ATP amplification in liquid suspension may not be suitable for biological drug products that require bioburden enumeration using the conditions tested**
  - Cannot monitor "total number" of microorganisms associated with a specific item per FDA Guidance
  - RLU does not provide meaningful microbial load measurement
  - Detection time ~24 hours
- **ATP amplification in liquid suspension may be suitable for biological drug products that require microbial testing using present/absent metric**
  - Agreement between alternative method and visual inspection for positive detection of test articles with initial low contamination level
  - Consistent detection time of at least 24 hours
  - Potential risk to product quality associated with testing before 24 hours

**Future direction**

- **Sterility performance using ATP amplification in liquid suspension still ongoing**
- **Evaluate performance of other commercially available ATP detection platforms**

## References


- Guidance for Industry Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice (2004)
- USP <61>, USP <71>, USP <1223>, USP <1071>
- Bugno, A., Almodovar, A.A.B., Saes, D.P.S. et al. Evaluation of an Amplified ATP Bioluminescence Method for Rapid Sterility Testing of Large Volume Parenteral. J Pharm Innov 14, 152–158 (2019). https://doi.org/10.1007/s12247-018-9344-y
- Jurtshuk P Jr.. Bacterial Metabolism. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Chapter 4. Available from: https://www.ncbi.nlm.nih.gov/books/NBK7919/
- Parveen S, Kaur S, David SA, Kenney JL, McCormick WM, Gupta RK. Evaluation of growth based rapid microbiological methods for sterility testing of vaccines and other biological products. Vaccine. 2011 Oct 19;29(45):8012-23. doi: 10.1016/j.vaccine.2011.08.055. Epub 2011 Aug 24. PMID: 21871516.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