Modern Adaptations to a Rapid Microbiological Method for Biologically Derived Products Using ATP-Bioluminescence

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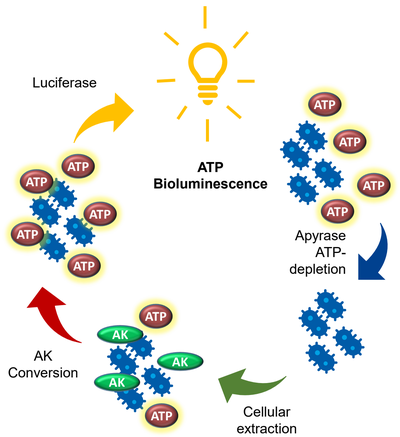
1 MECHANISM OF ATP ASSAY & DEPLETION

ATP-bioluminescence has been used for the detection of microbial contamination for many decades. While widely applied to different product-types, there are still challenges in terms of overcoming interfering factors. One challenge is the persistence of residual ATP from other sources, such as biologically derived sample-types. Residual ATP can contribute to bioluminescence during an ATP assay, even when the sample does not contain microorganisms.

Understanding the biochemistry of a given ATP method can support solutions for difficult products. Celsis AMPiScreen®, for example, relies on phosphate transport molecule, Adenylate Kinase (AK), to amplify ATP Bioluminescence by converting ADP to ATP, before deploying luciferase to activate bioluminescence. A new background depletion assay, **AMPiScreen® AP**, uses chemical depletion of ATP, and then ADP conversion, thus allowing an opportunity to deplete background ATP from samples before amplification and then detection

This poster outlines use cases for alternative ATP reagent chemistries and the study performed to demonstrate equivalency of this method to the traditional sterility test.



2 CASE STUDY 1: BIOLOGIC PRODUCTS

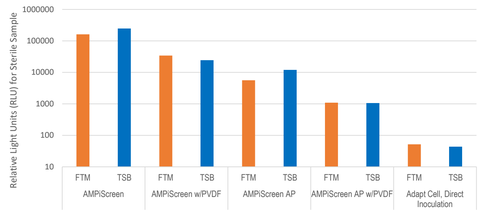
ATP-bioluminescence is highly reliable as a microbial test marker, since all microbiological contaminants produce ATP as part of their metabolism. However, ATP can persist in other substances as a result of other biological processes.

For example, egg-based vaccines, while not themselves cellular in nature, are derived from a biological process. Depending on manufacturing controls, residual ATP or molecules which phosphorylate ADP to ATP can provide a false signal for presence of microorganisms.

In this case study, a biological sample was tested in FTM and TSB sterility medias. Different treatments were performed to reduce background noise:

1. Conventional AMPiScreen®
2. Low binding filtration (PVDF) with AMPiScreen®
3. **AMPiScreen® AP**
4. Low binding filtration (PVDF) with AMPiScreen® AP
5. **Celsis Adapt™ Cell**

Table 1. Effects of Sample Treatments on ATP Bioluminescence



This study concludes that ATP-depletion, available in both **AMPiScreen® AP** and **Celsis Adapt™ Cell**, are effective at depleting ATP background derived from the biological sample.

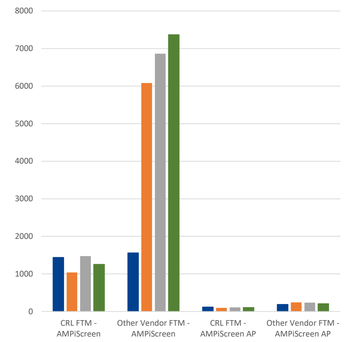
3 CASE STUDY 2: HIGH-ATP MEDIA

Another challenge in terms of high background is media control. Low ATP background is needed to discern very low levels of microorganism from the media baseline. Charles River addresses this challenge by providing ATP-controlled medias for Sterility and Home & Personal Care laboratories.

There are several reasons why this may not be the best solution for all laboratories. For example, these could be related to supply-chain challenges or internal laboratory workflow restrictions. Instead of switching media, a second solution could be to use ATP-bioluminescence assays which deplete background components from the media, thus reducing or eliminating the need to qualify specific media supplies which are compatible with the conventional AMPiScreen® assay.

In this example, four individual lots of Fluid Thioglycollate (FTM), a known high-background media, were analyzed using conventional AMPiScreen and **AMPiScreen® AP**. While Charles River-provided media is already low in background using the conventional AMPiScreen® assay, the alternative vendor media can exhibit backgrounds as high as 7000 RLU, which is incompatible with detecting low CFU in a contaminated sterility test. This example shows how AMPiScreen® AP reduces the background for the alternative vendor media such that it is comparable to Charles River media.

Table 2. Relative Light Unit (RLU) Background For Charles River FTM versus an Alternative Supplier



4 3-WAY EQUIVALENCE: COMPENDIAL STERILITY | ATP-BIOLUMINESCENCE | ATP-BIOLUMINESCENCE WITH DEPLETION

After exploring the effect of ATP depletion on ATP-bioluminescence assays, it is reasonable to study the effects of ATP depletion on microbial signal. While the reagent process deploys depletion before extraction, amplification, and bioluminescence, it is important to understand if the depletion reagent impacts ATP from low levels of microorganism, which must be detectable in a routine sterility test.

An equivalence analysis was therefore performed per USP <1223>, examining the non-inferiority of Celsis® assays to the USP <71> compendial sterility test. This also afforded the opportunity to examine detection differences between the conventional AMPiScreen® assay and the alternative **AMPiScreen® AP** Assay. The study design incorporates:

- 10 microorganisms, including
  - 6 USP <71> microorganisms
  - 3 environmental isolates
  - Stressed *C. acnes*
- 6 Sterisart™ sterility canisters per microorganism
- Inoculum of <5 CFU to each canister

In this study design, between 50-75% of total test replicates are expected to be positive. The plated inocula level was used to determine if either Celsis result was inferior to the USP <71> result.

The results found that the **AMPiScreen® AP** assay performed exactly the same as the AMPiScreen® assay for detecting low levels of microorganisms at <5 CFU. Both methods were found to be non-inferior to the USP sterility test when tested after 6 days of incubation.

Table 3. Number of Positives Observed from 6 Replicates (1 CFU) During a 3-Way Equivalence Test

	S. aur	*P. aer*	*C. spor.*	*C. acnes*	*B. sub*	*C. alb*	*A. bra*	*M. lut*	*M. hal*	*R. muc*
USP <71> Sterility Test	3	4	2	2	6	3	3	4	4	5
Celsis AMPiScreen®	3	4	2	1	6	3	3	4	4	5
Celsis AMPiScreen® AP	3	4	2	1	6	3	3	4	4	5

Further study found that the **AMPiScreen® AP** reagent method as a means for depleting high background and detecting low microbial levels in a sterility test were robust, rugged, and repeatable, as well as compatible with a wide variety of product types, sample preparations, and media suppliers. Additionally, the assay can be deployed on existing Celsis® instrumentation with minimal installation and performance checks. This minimizes workflow complexity when switching between different assay reagents on the Celsis® platform.

Overall, these case studies demonstrate how ATP-bioluminescence has evolved to overcome ATP-background challenges associated with various product matrices. While earlier generations of ATP-bioluminescence tests limited the scope of products that could be tested using this method as a rapid sterility platform, further research and understanding provides solutions not previously available.