Technical Whitepaper

The Science of 2nd Generation ATP Monitoring

Advances in technology have lead to the development of a number of rapid microbiological monitoring methods over the last few decades, including methods based on the detection of ATP (Adenosine Triphosphate). Historically, ATP-based methods were susceptible to interferences caused by various components in a water sample. Consequently, traditional ATP test methods have not been sustainably applied in water, wastewater and other industrial applications.

Developments in the reagents and methodology used have led to substantial improvements in the ATP test method. LuminUltra has developed a patented portfolio of next-generation ATP test methods designed specifically for fluid samples, which are typically laden with components that interference with enzymatic assays such as those that measure ATP. These new, 2nd Generation protocols and reagents were specifically designed to mitigate various types of interferences including suspended solids, dissolved solids, biocides and organics. This is accomplished using chemical treatment and/or separation to isolate microorganisms from interference.

This whitepaper will provide an overview of ATP testing and discuss challenges that are faced in order to ensure accurate testing.

Introduction – What is ATP and How Does ATP Monitoring Work?

ATP is the primary energy carrier for all life forms and can be found only in and around living cells. As such, the measurement of ATP concentration in a sample provides a direct measurement of biological concentration and health. ATP is quantified by measuring the light produced through its reaction with the naturally-occurring firefly enzyme Luciferase using a Luminometer. The amount of light produced is directly proportional to the amount of ATP present in the sample. The following formula below shows the reaction that occurs with ATP in the presence of Luciferase enzyme:

$$ATP + O_2 + luciferin \xrightarrow[luciferase]{Mg++} AMP + PPi + oxyluciferin + light$$

In any sample, there are two basic types of ATP present (refer to Figure 1):

- Intra-cellular ATP – ATP contained within living biological cells.
- Extra-cellular ATP – ATP located outside of living biological cells which was released from dead or stressed organisms.

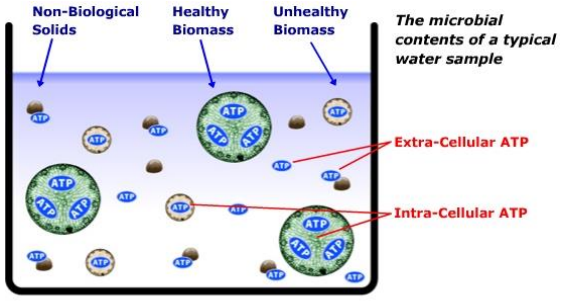

Figure 1: ATP Content of a Sample

It is important to accurately measure both types of ATP as well as differentiate between the two types to get a true assessment of microbial activity and health.

The Challenges of ATP Monitoring

Since the 1960's, the principles of ATP measurement have been successfully applied to a variety of applications, from environmental to marine monitoring as well as hygiene testing.

LuminUltra Technologies Ltd.
520 King Street
Fredericton, New Brunswick, Canada E3B 6G3
+1-506-459-8777
www.luminultra.com

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The latter application has been the most successful, in which the vast majority of food processing and distribution companies have adopted ATP methods as a quick test for total contamination under Hazard Analysis Critical Control Point (HACCP) strategies. These 1st Generation ATP test methods use basic principles and as such provided users with only a semi-quantitative measurement of ATP content. Due to their basic nature, these 1st Generation ATP kits have limited applicability.

The advantages of ATP testing over traditional culture tests are that the results are obtained in mere minutes (not days), that the results are complete (rather than the small fraction that will grow on culture media), and that the methods are generally field- and user-friendly. However, none of these advantages will provide any value unless the results produced are reliable and accurate. **This has been the Achilles Heel of 1st Generation ATP monitoring methods.**

As with any enzymatic reaction, the luciferase assay for ATP is susceptible to interferences that can be caused by various components in a sample. For this reason, ATP testing has not been sustainably applied in water, wastewater and industrial applications. In addition, ATP on its own is highly unstable and readily degrades to ADP (Adenosine Diphosphate) and AMP (Adenosine Monophosphate), unless it is complexed with other molecules or cell debris. As such, this presents several challenges to achieve accurate ATP measurement, including:

- **Ensuring that the method is designed to meet the unique needs of the sample**
  Traditional ATP tests are provided as a 'one-size-fits-all' package. LuminUltra has recognized that there are a great many unique sample types that demand different handling and therefore provides specialized kit formats to address unique situations.

- **Quantitative incorporation of the sample into the test**
  Good results start with good sampling and incorporation of that sample into the test. Fully quantitative methods are required to obtain accurate results.

- **Ensuring complete extraction of intra-cellular ATP**
  In order to get an accurate ATP reading, it is critical that ATP from all cells in a sample is recovered. It is especially difficult to extract ATP from biofilm clumps, microbial floc, yeasts and molds. As such, having a weak extraction reagent can lead to incomplete cell lysis and thus only partial ATP recovery yielding lower than actual results. Therefore, it is important to ensure that the extraction reagent used is strong enough to completely lyse all microorganisms present in a sample for maximum ATP recovery, while not being too strong such that it inhibits the luciferase enzyme. Striking this fine balance is a major challenge in accurate ATP monitoring, and one that LuminUltra has overcome through years of research.

- **Stabilizing all released intra-cellular ATP and as well as extra-cellular ATP prior to assaying to prevent its degradation between recovery and assay**
  The high-energy bonds in an ATP molecule make it a rather unstable molecule and when not stabilized, ATP readily hydrolyses to ADP and phosphate. Most extra-cellular ATP is complexed to various molecules and cell debris, and is unavailable to react with the luciferase enzyme. When intra-cellular ATP is extracted, it readily complexes or degrades, making it unavailable to react with the luciferase enzyme. If left unbuffered, the instability of an ATP extract can lead to lower than actual results since most ATP would have either degraded or complexed before the luciferase assay is performed. It is important to ensure that all complexed extra-cellular ATP and all extracted intra-

cellular ATP is stabilized for maximum recovery.

- **Ensuring sample components do not inhibit the luciferase enzyme.**
  Samples often contain components that inhibit or quench the bioluminescent luciferase reaction. When not dealt with, these quenching and inhibitory agents decrease light output from the luciferase reaction and luminometer readings are lower than they should be, giving users false results. Quenching and inhibition can come from various sources including sample colour, dissolved solids, suspended solids organics, heavy metals, biocides etc. Therefore, it is important that the effects of all of these various components that may be present in a samples are minimized or neutralized prior to the luciferase assay.

- **Minimizing outside influence in the final output of the test.**
  The output of the ATP test is light, which is measured in a luminometer and reported as Relative Light Units (RLU's). This result can be impacted by not only the quantity of ATP in the sample, but also the reaction temperature, the luminometer make, model and overall condition, the enzyme age, potency and concentration, among other things. LuminUltra accounts for these variables by converting RLUs to ATP concentrations using its liquid-stable ATP standard, UltraCheck™1. Using unconverted RLU values as final results is essentially equivalent to using an un-calibrated instrument.

Overcoming all of these challenges is the key to an accurate ATP monitoring protocol. When ATP monitoring has a number of benefits, the most valuable is its rapid turnaround time – it can take as little as five minutes from when a sample is obtained and processed to when a result is obtained, allowing users to work with almost real-time results. In addition to its rapid turnaround time, most ATP test reagents and equipment are portable allowing users to perform samples analyses at various sites or in a lab. Only if the results are accurate are these advantages exploited to their fullest potential.