Technical Whitepaper

Comparing 2nd Generation ATP to Culture-Based Test Methods

Introduction

Water is a vital resource for our planet as it is a requirement for all life forms, big and small, including microorganisms. As such, everywhere that water exists, microorganisms are sure to follow.

Fig. 1: Microorganisms are ubiquitous in our environment.

With the advance of industrialization, we have recognized the importance of monitoring and controlling microorganisms in consumption-based industries including manufacturing, pharmaceutical, energy, food processing and water treatment & distribution. If left unchecked, uncontrolled microbial proliferation can lead to potentially disastrous outcomes in process operations or for the individuals that consume contaminated products. Unfortunately, there is no shortage of examples where microbiological contamination of food, water, products or the environment have resulted in negative consequences.

Given the importance of controlling microbiological populations in industrial settings, it is surprising that the tools used to monitor it have remained largely unchanged throughout the years. Culturing techniques have been used as the standard method to identify specific microbes of concern in public and environmental health sectors for over 100 years. While these tools excel in identifying specific microorganisms, they have their limitations, particularly the speed of results (from 1 to 28 days or longer, depending on the species present) and the inability to quantify the total population (culture tests typically capture anywhere from 0.1-1% of the total population (Sloan et al., *The Uncountables*, Accessing Uncultivated Microorganisms: From the Environment to Organisms and Genomes and Back, 2008)).

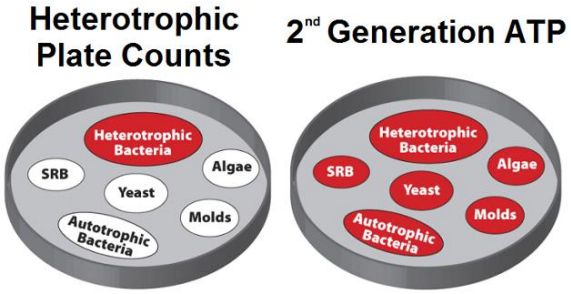

Fig. 2: HPC's only measure a fraction of the total microbial population compared to 2nd Generation ATP as indicated by the red highlights above.

LuminUltra's 2nd Generation ATP technology is an advanced method that fills the void in the microbiological toolbox and provides the first line of defense in your microbiological control program. These test kits are designed from the ground up to quantify the total microbial threat in any industrial fluid sample. The test is rapid (5 minutes), complete (quantifies all microorganisms in the sample), versatile (different test kits for different fluid types) and portable (can be utilized in the field).

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

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Considerations When Making the Comparison

Invariably, when new customers that are accustomed to the traditional culture methods begin using LuminUltra's solution, they want to know how well the two tests correlate. Overall, there will be differences between the results you report for culture tests and your ATP results owing to the mechanisms of action for each test. While the ATP test is a metabolic test, the culture test could be best characterized as a viability test; thus, it is not surprising that the two methods give different results since they are measuring different aspects of the microbiological population. While each tool is valuable for its intended purpose, one has to utilize the appropriate tool for the appropriate job. LuminUltra's 2nd Generation ATP test is the first line of defense since it is rapid and quantifies the total microbial threat. Utilize ATP testing to guide your mitigation strategies in real-time, allowing you to be proactive in your control program rather than reactive. Relying on culture tests to guide your mitigation program puts you at a distinct disadvantage since you will be dependent on process monitoring data that is already 24-48 hours old by the time you obtain results, and only tells part of the story regarding the total microbial threat. You must match the proper tool for the proper job: culture tests are great complimentary tools to quantitative ATP measurement, allowing you to identify certain problematic species that may be present within your system if that is a requirement for your operation. In many industrial settings, having information on specific species is irrelevant because it does not change the response or action taken in the face of contamination – the most common remediative action taken is to add a biocidal agent to the system, the vast majority of which are broad spectrum. Hence, in this case, the ATP test is the best qualifying tool to ensure that adequate treatment has been applied.

In general, culture tests and ATP tests correlate well for most sample types, and there is a directional relationship between ATP and CFU counts. Typically, ATP results will be higher relative to culture tests but there are exceptional cases where this may be reversed. Indeed, there are several key factors that affect the magnitude of the relationship and to say that there is a direct correlation between the two would be misguided as there are many different factors that will impact the relationship. Below we will review some of the more common phenomenon that will affect the relationship between ATP vs. CFU results.

1. Sample Heterogeneity

Perhaps the biggest source of variability in any microbiological test is heterogeneity within the sample. When microorganisms grow, they tend to do so in clumps or flocs, and as such, they are not evenly dispersed throughout fluid samples. This phenomenon is enhanced in complex industrial fluids that can have diverse chemical properties such as elevated viscosity, density and specific gravity. Moreover, these factors will affect settling time of the bacteria, which is inversely related to fluid viscosity and density. Put simply, how and where you obtain your samples and sub-samples will have great impact on the variability observed in your microbiological testing results. Furthermore, how this impacts your results is dependent on the test you are using. With culture-based tests, a clump of bacteria that may contain tens or hundreds of thousands of individual organisms will grow as one colony forming unit (CFU) on plates or dip slides, drastically underestimating the total population number. With the ATP test, all the cells in the clump will be counted and therefore the total population number will be much higher and more accurate.

Consistency in sub-sampling methods is key to minimizing variability in culture-based methods as well as the ATP test. The best strategy to reduce variability in results due to sample heterogeneity includes increasing your sample size (i.e. volume or mass), and by performing

more replicates. However, many culture-based methods such as dip slides use very small and unpredictable volumes for analysis (20-100ul), further increasing the variability and reducing the sensitivity of the assay. LuminUltra's test kits utilize a minimum of 1mL of sample to be tested and some methods enable users to scale that volume up to several hundred mL's, ensuring more sensitive and higher quality data that is more representative of the microbiological population.

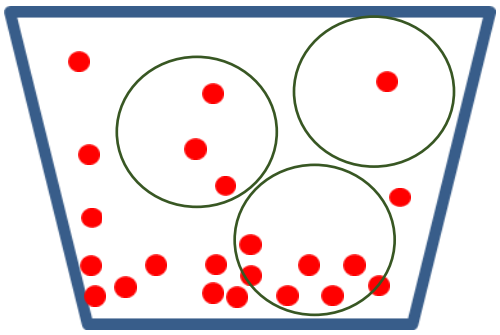

Fig. 3. In a typical industrial sample the dispersion of microorganisms will be asymmetrical. Therefore sample volume and number of replicates may have a significant impact on the quantification of organisms in the sample. In this example, 3 different test locations result in 3 different results (1, 3 or 6).

2. Culture conditions/effectiveness

Culture testing takes microorganisms from their natural environment and attempts to recreate a new artificial laboratory environment that is designed to be favorable for their growth and replication. However, the vast majority (>99%) of organisms from the environment will not grow under laboratory conditions (T.Kaeberlein, K. Lewis, and S. S. Epstein, *Isolating "uncultivable" microorganisms in pure culture in a simulated natural environment*. Science 296: 1127-1129, 2001). Several factors will determine the effectiveness of the culture, including the nutrients supplied, the incubation temperature, and other environmental conditions of growth (pH, pressure, oxygen levels, etc.). There are literally thousands and thousands of possible growth combinations that would need to be used to accommodate all of the potential organism's growth habits in the lab environment once removed from their natural environment. Additionally, the ability of microorganisms to be cultured depends on their current physiological state, and some microorganisms, such as Archaea, are extremely difficult to culture at all (DeLong E.F., Pace N.R., *Environmental diversity of bacteria and archaea*. Syst Biol. Aug; 50(4):470-8, 2001). Providing the proper nutrients and growth conditions for all of the potentially active microorganisms in an environmental sample is not feasible; therefore most of the active cells in culture samples will not grow in the chosen culture conditions, resulting in an underestimation of the total population. In fact, it has been reported that culture results typically only report 0.1-1% of the total population (Sloan *et al*., 2008). Owing to the limitations of the culture test as a condition monitoring tool, it is advisable to utilize other complimentary tools such as ATP that are rapid and complete to compliment the ability of the culture test to isolate specific microbes of interest in a sample (such as coliforms, or *E. coli*).

3. Microorganism Doubling Time

Most dip slides and petri plates incubate samples for 1-2 days and then count colonies to estimate the amount of bacteria or fungus present. Since it takes approximately 1 billion cells (~30 generations) to form a visible colony, counting colonies after 1-2 days of incubation presumes that all microorganisms have a doubling time of ~0.5 to 2 hrs. In reality, microorganisms can have generation times of many hours or even many days, and therefore these slow growing organisms will not get counted in the majority of culture tests. In populations comprised of both fast and slow growers, the fast growing organisms will quickly overgrow and mask the evidence of any slow growers before they can be detected, further skewing the quality of data obtained. Therefore, the organisms doubling time is a very important

component to what gets reported in the culture results and is often not considered.

4. Population Profile (Metabolically Active vs. Dormant Cells)

Microorganisms that inhabit industrial fluid samples are remarkably adaptable and can exist in many different forms. Healthy active organisms that are viable will make up a proportion of many fluid samples, but there can also be injured or slow growing organisms, fungal and bacterial endospores, as well as dormant microorganisms that are not currently active and healthy owing to insufficient growth conditions, but which could potentially re-activate given the appropriate environmental change. ATP will detect both the healthy and unhealthy organisms, the slow growers and the injured (but viable) population; however it will not detect the dormant population or spores as they will contain too little ATP to quantify. For this reason, in certain situations, culture tests may actually over-estimate the active population by allowing dormant and spore populations to be re-activated in the rich growth media supplied in the lab environment that would not otherwise be active or potential threats in the natural environment.

5. Biocide Considerations

Biocides are used to control the microbiological population and their use will affect how results are interpreted for both ATP test and culture tests. For example, a culture-based test will not be able to distinguish between lysed cells, dead but intact cells (i.e. organisms that are metabolically inactive but have not lysed), and inhibited cells (i.e. cells that are alive but cannot reproduce). Because nearly any biocidal treatment will produce all three types of cells, culture-based tests will tend to overestimate biocide performance and/or microbial kill. By their nature, culture-based tests are more sensitive and specific to target populations but require longer time periods to render results when compared to metabolic-based tests. On the other hand, ATP tests are metabolically based and will quantify inactivated cells that remain intact. This could include VBNC cells, stressed or injured cells that remain intact and inactivated (preserved) cells that remain intact. Owing to the completeness of the assay, the ATP test will tend to underestimate the effectiveness of biocide treatments. In other words, culture-based testing will give an optimistic view of biocide performance while ATP will provide a more conservative estimation.

In addition, the use of biostatic agents will inhibit the growth and replication of microorganisms without actually killing them. In this instance, when culturing samples with biostatic agents, once the agent is diluted past a critical threshold it will no longer inhibit growth and will allow colonies to form, over-representing the active population due to the release of its inhibitory effects. For more detailed information on how to interpret and plan biocide treatments, please consult our **Kill Study Guidelines** document.

Comparing Low Detection Limits in ATP vs CFU tests

Healthy organisms that are actively metabolizing will contain sufficient ATP to perform important cellular functions. An average *E. coli*–sized bacteria cell will contain approximately 1 femtogram (fg) of ATP, while the average fungus contains 10 to 100 fg ATP/mL. Utilizing this information we can estimate the low-detection limit of LuminUltra's 2nd Generation ATP test kits using CFU/mL as the basis for comparison. Using the QGO-M test kit as an example, the following table illustrates how the ATP test will compare with culture-based tests at the low detection limit, dependent on the % culturability of the sample:

Table 1 – Comparison of low detection limit for LuminUltra's QGO-M test kit compared to culture-based tests considering the % culturability of the organisms in the sample.

%	Bacterial	Yeast/Mold

Culturability	CFU/mL Low Detection Limit	CFU/mL Low Detection Limit
100	1,000 CFU/mL	10 CFU/mL
10	100 CFU/mL	1 CFU/mL
1	10 CFU/mL	< 1 CFU/mL
0.1	1 CFU/mL	< 1 CFU/mL
0.01	< 1 CFU/mL	< 1 CFU/mL

QGOM – Low Detection Limit of ~1 pg/mL = 1,000 fg/mL (using 20mL of sample)

As previously discussed, the majority of culture tests only report 0.1-1% of the total population due to the selection pressures generated by the artificial growth environment for the microorganisms to grow. Therefore, with these typical numbers in mind, the low detection limit for LuminUltra's QGO-M test kit is equivalent to between 1-10 CFU/mL.

Summary

As discussed, for many applications there will be a directional correlation between CFU data and ATP data, while the magnitude of the relationship will oftentimes vary for many of the reasons presented above. Most typically this is because of the nature of the differences between the two tests: LuminUltra's ATP test is a metabolic test, whereas culture tests will measure viable cells. In cases where ATP data does not correlate well with CFU data, you must consider the sample type and characteristics to properly interpret results. Examples of some common situations that cause the two results to not correlate well:

Factors that cause High ATP but Low CFU:

- Clumped biomass.
- Non-culturable microorganisms.
- Organisms that are damaged and cannot be cultured.
- Slow growing/doubling time organisms
- Negative interference in the CFU test from residual biocides

Factors that cause Low ATP but High CFU:

- Positive interference (i.e. external contamination) in the CFU test.
- Spores and other dormant cells that get re-activated in rich nutrient broth.
- Negative interference in the ATP test.

Overall, these tests are very different but also each is highly useful when put to use in the appropriate way. As such, culture tests and ATP should be considered complimentary, **not** competitive techniques, and each should be leveraged to provide the most useful data for your microbiological control program.