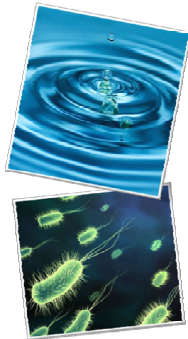


Background – Understanding the Objective

Everywhere that water exists, microorganisms are sure to follow, and the risks associated with microorganisms in water and other fluids are well-known across all industries. There are a variety of different measurement techniques that have been developed to assess microorganisms, but until very recently there have been very few tools available that had wide-ranging applicability to multiple



applications and to multiple users. As a consequence, the traditional tools are often utilized for sub-optimal purposes rather than their specific niche.

Traditional microbiological tests such as culture testing and microscopic examination have existed since the 1800's. An update to the overall microbiological toolkit is long overdue. 2nd Generation ATP testing represents a major upgrade over previous 1st Generation versions. But in the context of the overall requirement for microbiological testing, 2nd Generation ATP testing is advantageous for three primary reasons:

1. It is **fast** - results are available in a matter of minutes rather than days. Obtaining near real-time results ensures that you are aware of any impending threats immediately, rather than having to wait days for results to be available via culture tests or for reports to come back from remote laboratories. This allows corrections in growth control programs before serious problems develop. Furthermore, this speed allows you to re-test if you

obtain erroneous results, which is not possible with other types of microbiological tests.

2. It is **portable** - you can take the microbiology testing laboratory to the field. Being able to perform testing right at the heart of the process eliminates any concerns about sample properties changing en-route to the laboratory, and it also offers you the opportunity to trace threats to their source while you are at the site. In short, the combination of speed and portability allow for on-the-spot problem solving, shortening troubleshooting cycles to a matter of minutes or hours versus traditional days or weeks.
3. It is **complete** - because ATP is present in all living cells, you will be assured to measure the total 'threat'. This is significant when you consider that when microbiological threats are identified, the typical responses (anti-microbial initiatives, including chemical disinfectants, non-chemical disinfection, pasteurization, filtration, and so on) actually attack the total microbiological population. Hence, a complete measurement such as 2nd Generation ATP is the best confirmatory tool for remediative treatments!

In short, the 2nd Generation ATP test fills a need that has long existed in the microbiological tool kit and actually complements the other methods that have become commonplace in industry. It is the first line of defense in identifying threats, after which follow-up testing to speculate the problem can be undertaken if necessary.

One of the most frequently-posed questions that we field here at LuminUltra is, "**How does 2nd Generation**

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ISO 9001

ATP testing compare to my existing microbiological monitoring practices?" To see how LuminUltra's test kits stack up against the competition, read on!

1st Generation ATP Test Kits

These types of methodologies have been commercially available for many years and are used quite successfully in food and medical surface hygiene applications. In brief, swab-based all-in-one devices are provided by multiple suppliers around the world that provide a semi-quantitative, "go/no-go" style confirmation of surface cleanliness. In some instances, these same suppliers have adapted their products to water applications by the substitution of the swab for a 'dip stick'. These products have only limited application and do not provide the required degree of quantitiveness for reliable process control in water and other fluid applications. In brief, the advantages of 2nd Generation ATP tests over their 1st Generation counterparts include:



1. **Sampling Accuracy** – ensuring a known quantity that is sufficiently large to meet sensitivity requirements significantly improves accuracy and precision of the method.
2. **Complete Extraction** – 2nd Generation methods maximize ATP extraction, whereas competing products only extract and measure a fraction (generally 30 percent or less).
3. **Resistance to Interferences** – The biggest difference that sets LuminUltra apart from 1st Generation ATP test kit providers is that all other ATP testing products are adaptations from those used in food and hygiene applications and for that reason they are often distorted by interferences (i.e. chemicals, metals, etc.) Rather than following the same path, LuminUltra re-invented this concept so that it would be far less susceptible to interferences and therefore suitable for use in all fluid applications.

4. **Quantitiveness** – All 2nd Generation ATP test kits come with an ATP standard (UltraCheck™ 1) that converts instrument outputs into ATP concentrations and subsequently into approximations of microbial counts. This not only puts results on a more easily understood basis, but it also accounts for instrument quality, enzyme activity, temperature, and several other factors. Converting RLU results using the UltraCheck™ 1 standard enables LuminUltra's ATP tests to be used with nearly any luminometer that uses a photomultiplier tube.
5. **Compensation for Dead Cells** – 2nd Generation methods can isolate living microorganisms from dead ones. Conversely, competing ATP test methods usually do not differentiate, and in the rare cases that they do, they require two separate tests (doubling the cost per test).
6. **Application-Specific** – The 2nd Generation line of ATP test kits includes kits designed for specific types of samples rather than a "one size fits all" solution, ensuring that samples are processed according to their specific and unique characteristics to ensure the most accurate results.

1st Generation ATP testing methods are largely unsuitable for fluid-based applications. For more details on how 2nd Generation ATP methods improve on 1st Generation methods, please see the document entitled '*The LuminUltra Difference – How 2nd Generation ATP Technology Improves on 1st Generation ATP Test Methods*'.

Microscopic Examination & Enumeration

One of the most tried and tested methods of diagnosing microbiological contamination and / or proliferation is the microscope. In the hands of a well-trained user and given adequate equipment and resources, the microscope remains the standard for determining the extent and/or diversity of microbiological issues in process operations. Studies



have shown that the results from 2nd Generation ATP testing compares extremely well to microscopic enumeration techniques, especially when Live/Dead stains are used to enhance the precision of the latter¹. If the concentration of non-biological components is much higher than biological components, then viewing biological components is obscured. Furthermore, the detection limit for microscopic analyses can be higher than required for most applications – with modern equipment, this limit is generally 1,000 cells. Finally, the cost for adequate microscopy equipment and supplies can be quite high, not to mention the training required to ensure they are used to their maximum potential.

Overall, 2nd Generation ATP testing is quite complementary to microscopic examination, in that the former is ideally suited for field use to diagnose microbiological problems which can be further examined by the latter in a controlled environment.

Culture Tests

Perhaps the most common method of microbial enumeration is the Culture Test. Types of culture tests include the **plate count**, the **dip slide**, **serial dilution bottles**, and colorimetric growth-based methods like the **Biological Activity Reaction Test** (or **BART**).



These methods operate on the principle of growing microorganisms in colonies until they can be visually enumerated (i.e. counted by the naked eye). They remain the best and most reliable means to quantify the existence of viable microorganisms that are culturable in a certain environment. However, they have the disadvantage of long sample incubation as well as careful preparation requirements – you must select exactly the right media (nutrients), incubation temperature, atmosphere (oxygen versus no oxygen)

¹ Dockens et al., *New-Generation Microbial Testing Compares Favorably With Traditional Technologies in Identification of Bacterial Contamination of Oil and Gas Systems*; International Coal Bed Methane and Unconventional Gas Symposium, 2010

and incubation time to quantify the target population. The user must also be very careful to not introduce external contamination into the test, and once the test is complete, the biohazardous microbial culture must be properly disposed.

Even with all of the right selections and precautions, there is no guarantee you will be able to quantify the target organism if they are in a **Viable But Not Culturable (VBNC)** state or if they are simply not capable of growing in the provided conditions. It should also be noted that a culture test will count a single, free-floating cell as one colony as it will for a clump of many thousands of microorganisms. Furthermore, there are still a great many microorganisms that we do not know how to culture (such as the domain Archaea). For these reasons, culture tests can significantly underestimate the total microbiological population². There is also a risk that your selected dilution will result in an unreadable result due to overgrowth, and often many dilutions must be performed to eliminate this possibility which increases both the labor and material cost of the test.

Also note that traditional culture tests are generally only used to test water or solids that are re-suspended in water, whereas 2nd Generation ATP testing can be used to analyze essentially any fluid sample as well as solids with no requirement for re-suspension. That is, ATP analyses are conducted without changing the environment of the microbial population.

Read on for more discussion about culture tests.

The Heterotrophic Plate Count

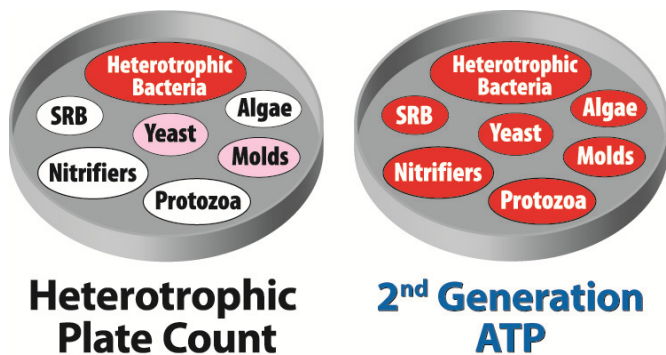
One culture-based method that is often considered to be in direct competition with the ATP test is the Heterotrophic Plate Count (**HPC**). These methods (sometimes referred to as Total Counts, Total Viable Counts, Standard Plate Count, or Total Flora) are a form of culture test that measure heterotrophic microorganisms (i.e. those requiring organic carbon or nitrogen as well as oxygen).

As with the 2nd Generation ATP test, these methods do not provide an indication of the types of organisms

² Sloan et al., *The Uncountables, Accessing Uncultivated Microorganisms*, ASM Press, Washington, DC, 2008, p. 35.

present or their sources. Consequently, they have been used as a means to estimate the total bioburden in a sample for a great many applications. Such a measurement has great benefit for process control, but the HPC falls short of this goal primarily due to the fact that it requires a minimum of 24 hours to obtain feedback, but also because it does not actually count the total microbial population.

The results obtained using an HPC test are not necessarily an accurate assessment of total heterotrophic concentrations but, instead, are indications only of culturable organisms present. For example, it has been shown that only approximately 1% of the total bacteria found using direct microscopy are enumerated using HPC procedures³. Possible explanations for this difference include the presence of some bacteria in a VBNC state and the fact that HPC media do not provide the complex nutritional requirements necessary for growth within the provided incubation time at the provided incubation temperature. This is to say nothing of the non-heterotrophic microorganisms that are present in a typical sample that would go un-detected in this test.



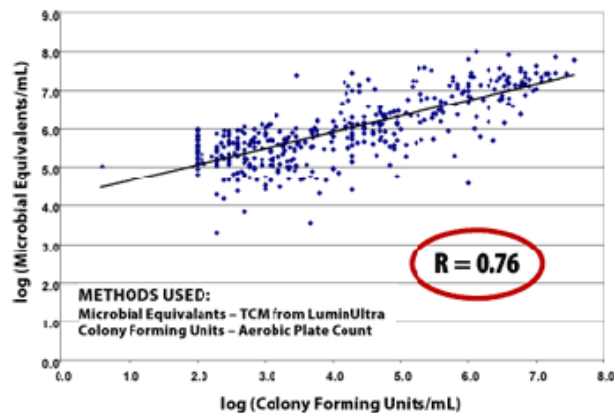
In spite of these differences however, there will often be a good correlation between results from 2nd Generation ATP and HPC measurements in many applications. This is because for most processes, microbiological diversity is relatively consistent. In fact, a significant deviation from an ATP to HPC correlation can signal a significant process change requiring investigation.

³ See Reference 2.

Therefore, the HPC method can be considered to be ill-equipped as a competitor to the 2nd Generation ATP analysis for the purpose of quantifying total microbiological concentrations. It is better thought of as complementary, providing information specific to heterotrophic microorganisms.

Correlating ATP Results with Plate Counts

The interest in conducting comparison studies between plate count and ATP results is understandable. Plate counts have been the benchmark to determine the degree of microbial contamination and to assess biocide performance for many years. In most situations you will see a decent correlation between ATP (which measures total microorganisms) and Heterotrophic Plate Counts (the de-facto standard plate count for estimating the total population).



There are three conditions that will act to improve the correlation of ATP measurements with any type of plate count or culture test, presuming the ATP test is fully quantitative and accurate:

1. When all viable organisms in the sample are measurable by the plate count.
2. When the ratio of the organisms not measured by the plate count are in the same ratio as the viable total population.
3. When the correlation between plate count and ATP is made over a large range of concentrations (at

least 3 orders magnitude – the greater the range, the greater correlation is improved. Disagreements of at least 10 fold can be obscured).

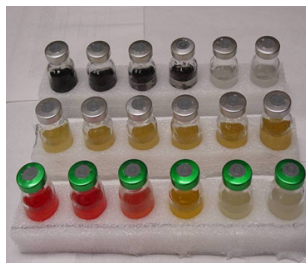
In other words, if the distribution of the population remains consistent at all times (i.e. the ratio of culturable microorganisms to total microorganisms remains constant), you will see a good correlation. When the correlation between these methods is not good, it generally involves situations where the population distribution is ever-changing, and the specific fraction measured by the culture test is changing in spite of little change in the total population. Therefore, although plate counts have been the standard to assess the amount microbial contamination at a site, any lack of correlation with ATP is not a suitable basis to reject the use of ATP monitoring.

Although monitoring content with plate counts has the two disadvantages of slow feedback and incomplete measurement, discontinuing their use is **not** advocated, either. Plate counts can provide a useful indication of population diversity (high diversity may indicate inadequate biocide application) and can also be used as a quality control check on ATP measurement.

Measuring both parameters is especially important in the initial phase of implementing a new microbial growth control program. At this initial phase, other tests for specific microorganisms may also be useful. After the program is established, the frequency of plate count and/or other types of monitoring can be reduced to reduce costs.

Culture Tests in the Context of “Kill Studies”

It is important to note that while the measurement of ATP can provide a similar directional response compared to culture tests, the overall magnitude of microbial reduction will not be as great for ATP-based tests. This is due to the fundamental differences between the test methods, described as follows:



- **Culture-based tests** rely on the growth of microorganisms in a nutrient-rich environment for quantification of microorganisms. Although

designed to replicate a natural environment for the test microbes, these artificial environments introduce selective pressures not normally encountered by indigenous biomass, including difference in carbon source, salinity, temperature, gas pressures. All of these factors play a role in influencing the growth of biomass. Additionally, a culture-based test does not detect cells that are alive but cannot reproduce under the culture conditions. 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 incubation periods to render results when compared to metabolic-based tests.

- **Metabolic-based tests**, such the measurement of ATP, generally involve a direct count of a target molecule. Because ATP is common to all forms of life, its measurement will yield an indication of total microbial community size – that is, anything that is metabolically active will be counted in the test. It can also count cells that have been inactivated but remain intact – although in this case, the ATP content of the cell is often dramatically reduced. In short, any ATP-containing cell will be counted in the ATP test, including viable cells capable of re-growth, viable but not culturable (VBNC) cells, stressed or inhibited cells, and metabolically-inactive but intact (preserved) cells. Because of its inability to distinguish between these various segments of the microbial community, ATP-based tests will tend to **underestimate** biocide performance and/or microbial kill. The primary advantage of ATP-based tests are their ease of use, portability, and extremely fast response (5-10 minutes), making them an ideal field tool as well as an excellent complement in the laboratory.

Whereas a successful biocide kill may be judged via culture testing to be a 4-log drop (i.e. from 6-log to 2-log, or from 4-log to 0-log), the equivalent drop in ATP will not be nearly as significant. Typically, a successful kill with ATP testing is a 2-log drop, perhaps even 3-log in some cases. This is primarily due to the elevated sensitivity of ATP testing to non-culturable organisms.

Overall, the perceived effectiveness of a biocide depends on a variety of factors, including dosage, the method of kill, its reaction speed, incubation or contact time. All of these factors should be considered along with the factors presented above about the monitoring tools utilized when setting up such a study. Exclusive use of plate counts has serious disadvantages in biocide screening. In addition to the problem of slow feedback, plate count tests provide no information about the effectiveness of the biocide treatment on the organisms they do not measure. Furthermore, they can be misleading if a biocide fails to penetrate a clump of microorganisms or alternatively disperses the clump.

For these reasons, any holistic kill study should strive to include a mix of culture-based and ATP-based monitoring to quantify biocide performance. As described above, using both tests helps reduce the shortcomings of each monitoring parameter. With that said, so-called ‘quick and dirty’ studies can be done in the field with ATP test kits alone assuming that a proper routine monitoring program is in place for follow-up.

Using Culture-based Tests for Process Control

As with any physical or chemical property, the control of biological threats in fluid processes requires a monitoring parameter – as the old saying goes, “*If you don’t measure it, you cannot control it.*” Because of its long history of use and wide variety of options, the culture test has become the process monitoring tool of choice to measure and control against biological (and more specifically, microbiological) threats. However, these methods are far from the best choice as a process control tool – rather, they are more suitable as a means of confirming that the process is meeting established performance criteria.



Effective process control against microbiological threats requires three critical characteristics:

1. **Speed** – Results must be available sooner (i.e. minutes or hours) that are available via culture methods (i.e. days or weeks). This is because

microbiological problems are most effectively and economically addressed in the early stages of growth. As microbial contamination load increases, higher concentrations of biocides are required for control and the potential for development of resistant populations is increased. Furthermore, speed would allow samples to be re-tested for confirmation if questionable results are obtained, which is not possible with tests that have significant time lag between initialization and bearing results.

2. **Portability** – The test method should ideally be able to be performed anywhere. Being able to perform testing at the heart of the process eliminates any concerns about sample properties changing en-route to the laboratory, and it also offers the opportunity to trace threats to their source in real-time. In short, the combination of speed and portability allow for on-the-spot problem solving, shortening troubleshooting cycles to a matter of minutes or hours versus traditional days or weeks.
3. **Completeness** – Consider that when microbiological threats are identified, the typical responses (microbiocidal treatments, chemical disinfectants, non-chemical disinfection, pasteurization, filtration, and so on) actually attack the total microbiological population. Only in certain cases does knowing the type of threat dictate the required action (e.g. deciding between a bactericide or a fungicide in the management of metalworking fluids). This means that the best follow-up measurement to quantify treatment effectiveness (again, in near real-time) should measure the total population. The use of the Heterotrophic Plate Count as a process control tool for this purpose is a very common approach. It is the most complete of any culture-type test in estimating the total population, making it the best-suited culture-based analysis for process control purposes. However, it still misses a significant proportion of the population and as detailed in the previous section, may overestimate the effectiveness of remediative treatments.

To enhance process control, a first-line test that meets the above criteria is required – this is the role that the 2nd Generation ATP test can play. Confirmatory culture tests (e.g. *E. Coli* measurements in drinking water applications, or *Legionella pneumophila* measurements

in cooling water samples) are an excellent means to confirm that indeed the process is in good control. In fact, a well-established process control program using 2nd Generation ATP can enhance your ability to ensure that culture test results for specific microorganisms are within acceptable limits!

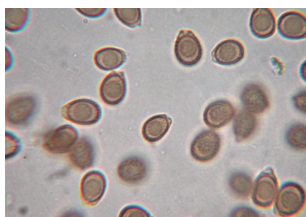
Impedance-based Monitoring Methods

Impedance monitoring to measure microbial contamination is a well-established technology that is used extensively in clinical, pharmaceutical, and food industries. It is a culture-based technology which uses the principle that microorganisms tend to produce by-products that decrease the electrical impedance of the culture medium when they grow and degrade the food in the medium. The rate of decrease is proportional to the size of initial microbial population.

Impedance monitors improve on traditional culture tests via decreased labor per test and potentially shorter incubation requirements to obtain results. However, they also share many of the deficiencies of traditional culture tests when it comes to fluid testing applications and especially in the context of process control. Economically, these methods are very expensive and are only suitable for use in laboratory environments.

Use of Culture Tests for Measuring Spores

In addition to their major advantage in quantifying specific microorganisms, culture tests are the only truly effective means of quantifying non-vegetative spores. Spores – which are the dormant forms of certain fungi and bacteria – exist in a state of suspension and therefore have very little metabolic activity. This means that they contain far less ATP than a vegetative (active) cell. They are most effectively detected via culture testing, or by re-activation prior to an ATP measurement.



A key difference between ATP and culture tests as microbial enumeration methods is that ATP measures all microbial cells (both viable and non-viable) whereas culture tests measure only viable cells. Also, ATP measures ALL microorganism types, whereas culture tests measure only the microorganisms that are capable of growing in the environment provided. It is generally accepted that typical culture tests will recover 0.1% - 1% or less of the total population, and some newly-discovered microorganisms (such as *Archaea*) cannot be cultured at all. Hence, culture tests underestimate total microbial population size. Additionally, ATP results are available in minutes versus days or weeks for culture tests. In some cases, colony formation can take longer than the suggested incubation time and if checked early will show no growth giving false results.

The ATP test and culture tests are very different tools and should **not** be thought of as mutually exclusive. Rather, culture tests are like microscopic examination methods in that they are complementary to the 2nd Generation ATP test – they measure microorganisms in different and highly useful ways. Such information can be exceedingly useful for example in determining the source of a specific threat, or to ensure that health and environmental risks are minimized. The reason that these tests are sometimes viewed as being competitive to the 2nd Generation ATP test is because they have long been the best option available for meeting our requirements. But now with new technology available, there is a better way.

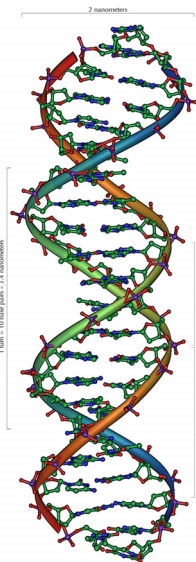
Molecular Biology Methods

Development of test methods based on genetic analyses dominates current research, but such methods are commercially very new. The most common types include **PCR** (Polymerase Chain Reaction), **DGGE** (Denaturing Gradient Gel Electrophoresis), **FISH** (Fluorescence In-Situ Hybridization), and **MDM** (Microbial Diagnostic Microarrays, also known as “Gene Chips”). These types of analyses are likely to revolutionize the microbiological testing field in coming years. Such test methods currently require controlled laboratory environments, skilled operating staff, and significant capital and operating investment.

In fact, these methods are quite similar to ATP measurements in that they measure molecules

Summary

associated with microorganisms rather than traditional methods that quantify or qualify the microorganisms themselves. The basic principle of genetic analyses is the detection of the presence of specific chains or sequences of genetic material. These chains may be related to a gene or to RNA produced from gene. In this case, the capability of making a gene product (protein) is inferred from test results. If the gene is unique to a kind of organism, then the presence of the organism is also indicated by the test. Alternatively, the chain of genetic material may simply be a region of DNA or RNA that is specific to an organism or is specific to a group of organism types. In this case, the presence of the organism(s) is inferred by the test



These methods are extremely useful when 'searching' for specific types of microorganisms, and are likely to compete with culture-based analyses for this purpose in coming years due to their improvement on certain shortcomings of culture-based tests. Current research is focused on improving their ability of these tests to distinguish between living and dead organisms, and improving their ability to resist interferences in typical fluid samples. Regardless of these advancements however, these test methods will always remain complementary to the ATP test in that they each serve different purposes.

Immunoassays

An immunoassay is a biochemical test that is based on the unique ability of an antibody to bind with high specificity to one or a very limited group of molecules, called an antigen. These are generally specific to a certain type or class of biological cells.

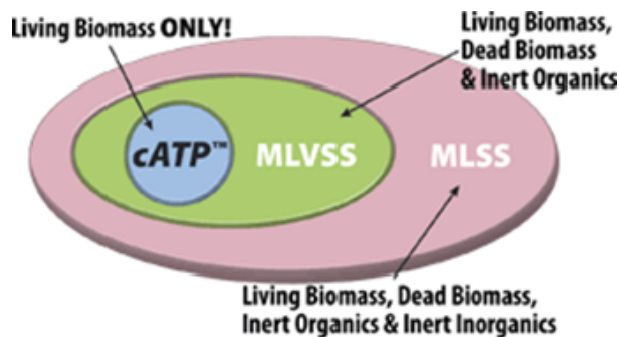
Like other molecular biology methods, Immunoassays are a relatively new method that has significant promise and is likely to be utilized more and more in the industrial setting in coming years. They are again complementary to 2nd Generation ATP testing due to the

fact that they measure for specific segments of the total population.

Particulate Analyses

The quantification of particles in a fluid is used extensively in fluid management applications. Specific methodologies include the measurement of Total Suspended Solids (TSS), Volatile Suspended Solids (VSS), Turbidity, and more recently Particle Counting and Particle Size Distribution. Some technologies even make use of light reflection patterns resulting from the scattering of lasers to classify particles. However, all of these approaches are unable to directly and reliably distinguish between living, dead, and non-biological solids and are therefore prone to large positive interferences when used to specifically detect microorganisms.

Wastewater treatment systems often use suspended solids to infer the concentration of living biomass contained in a bioreactor. However, these measurements include living and dead microorganisms as well as inert particles. By contrast, ATP monitoring provides a direct measure of the living portion of the bioreactor and hence provides the user with a clear picture of the engine that drives the process – the biomass itself!



A routine ATP monitoring program provides superior insight into the biomass health and activity than solids measurements, and as such, represents the ideal process control and continuous improvement tool for wastewater treatment monitoring.

Bioassays

This class of measurements analyzes the response of an organism to some condition imposed upon it. Most often, they are used to quantify any toxicity associated with a water sample. The most common of these assays include the **Rainbow Trout Toxicity Test**, in which the response of these fish is measured as the percent remaining alive after a period of exposure to a target water sample.



More recently, the **Microtox®** assay has been used for the same purpose. Rather than using a specific type of fish, this test method uses the light-producing (i.e. bioluminescent) bacteria *Vibrio fischeri*, and operates on the principle of less light production meaning greater toxicity.

These measurements are very different from the rest of the techniques described in this document in that they do not quantify microorganisms in general or by specific type; rather, they quantify the impact of a condition on a surrogate organism as supplied in the test.

As a means to quantify potential environmental impact of wastewater discharges, these methods are well-accepted and reasonably accurate. When it comes to quantifying toxicity of untreated wastewater entering a bioreactor however, the methods described above are less than ideal, due to the fact that they utilize surrogate organisms in place of the indigenous organisms that will actually face the toxicity. This can lead to incorrect prediction of toxicity, either as false negatives or false positives.

Conversely, a routine ATP monitoring program provides the **Biomass Stress Index (BSI)** parameter – a direct indicator of acute stress on process biomass. By using the BSI to observe the target population's response to various stimuli (good and bad), the user can immediately detect poor biomass health. As such, not only can process upsets be detected and prevented, but unfavorable conditions such as low oxygen or suboptimal pH can be identified and remedied.

Respirometry

A respirometry test is defined as a procedure used to determine the metabolic activity of a microbial population. For aerobic processes, this is generally done by measuring the rate of oxygen uptake of a small amount of biomass incubated in a large quantity of process influent. Common methods include the **Biochemical Oxygen Demand (BOD)** and **Oxygen Uptake Rate (OUR)** test methods. For anaerobic processes, a similar procedure is used where carbon dioxide production is monitored.



Hence, the basis of respirometry is the measurement of gas consumption or production from a microbial population. Because gas consumption or production is directly tied to metabolism, respirometry provides direct information on the consumption and/or inhibition rate of biological degradation of chemical substrates. By comparison, 2nd Generation ATP in a bioreactor sample provides direct measurement of biomass characteristics, including:

- The energy available to the population to do work (via **Cellular ATP**, or **cATP**);
- The integrity of the cell membranes (via the **Biomass Stress Index**, or **BSI**);
- The relative proportion of bulking floc (via **Floc-Bulking ATP**, or **fbATP**).

Although some of the applications for these two measurements have similar objectives, it is clear from the above that the tests are fundamentally different. Because of the complex nature of biological processes, viewing the objectives from different perspectives increases the power of both analyses. On this principle alone, it is beneficial to use both technologies to monitor a bioreactor.

It is likely that ATP-based parameters can provide certain insights better than respirometry, and vice versa. However, it is important to note that a series of ATP measurements on an incubated sample can provide information similar to a respirometry test – since ATP is

a measurement of energy, a series of discrete measurements will define the activity of a sample. In addition, because ATP provides an estimate of active biomass that is superior to TSS and VSS, the two tests can be directly integrated to provide a more accurate SOUR (Specific Oxygen Uptake Rate), i.e. oxygen uptake rate per unit of biomass (cATP), or OUR divided by cATP.

Furthermore, for applications related to measuring BOD levels throughout the process, the ATP assay is extremely useful to standardize test conditions by ensuring that the same quantity of active biomass is used in each respirometry test. In performing any respirometry or incubation analysis, it is important to balance the amount of biomass and process influent mixed together. A small relative amount of biomass can shorten the time required to identify positive or negative responses, but can also result in false positives for toxicity detection. Conversely, if the amount of biomass used is too high, false negatives for toxicity can result. The use of the ATP test in concert with respirometry measurements can mitigate this concern.

LuminUltra's **Biomass Growth Index (BGI)** protocol can also provide superior information to a respirometry test in terms of direct biomass response. A series of discrete tATP and dATP measurements done on an incubating sample yields information on the rate of change in energy (cATP) and stress (BSI) of the biomass. This can allow the operator to distinguish between changes in biomass concentration (i.e. growth or kill) and biomass stress.

The following is a series of comparative points for the two technologies.

- **Advantages of ATP Compared to Respirometry:**

- Provides direct information on biomass energy and health within minutes;
- High sensitivity permits monitoring of settling process;
- High sensitivity permits more sensitive monitoring of toxic and inhibitory substances because smaller levels of biomass can be used in the toxicity assay (e.g. the BGI test);

- More sensitive to chronic stresses such as food and nutrient deprivation;
- Capital expenses do not increase as monitoring intensity increases;
- Less maintenance (because everything is disposable).

- **Disadvantages of ATP compared to Respirometry:**

- More labor for BGI-type test;
- Each measurement has reagent and disposables costs.

- **Advantages of Respirometry Compared to ATP:**

- Continuous measurements ensure that precise lag and peak gas production / consumption is detected (respirometer vs. BGI test);
- No additional labor for many measurements over an incubation period (respirometer vs. BGI test);
- Provides direct measurement of gas consumption activity.

- **Disadvantages of Respirometry Compared to ATP:**

- Expensive to compare many conditions simultaneously (DOUR, respirometer);
- Respirometry is slower to detect stressed populations and stressful environments. DOUR may not detect a stress until major damage has occurred within a population;
- Biomass concentrations required for detection are more than 1000 fold higher.

The measurement of the rate of oxygen consumption or carbon dioxide production is an important operating characteristic for aerobic and anaerobic bioreactors, respectively. Although some of the applications for the two technologies have similar objectives, it is clear that the tests are fundamentally different.

Chemical Methods

Chemical methods are defined as those that measure for specific chemical characteristics of the sample. In the context of microbiological threats, these methods are used to **infer** the presence or absence of microorganisms since they cannot directly quantify biological cells. They are therefore excellent complements for a holistic monitoring strategy, but do replacement microbiological testing methods. Specific types of analyses are discussed as follows.

Biocide Residual

Municipal and industrial operators are taught that so long as a biocide residual is maintained in a water system, good microbiological control will ensue. While this is largely true, it is not always the case.



For instance, a measurable biocide residual in a bulk fluid is confirmation that for that particular sample, there is a low probability of microbial survival. Assuming this to be the case for the rest of the system – whether it is a well-stirred tank or an extensive water distribution network – is a tall assumption. Furthermore, maintenance of a residual for an oxidizing biocide such as chlorine speaks to a stronger potential than for non-oxidizing biocides for which microorganisms may build a resistance, although sessile or attached biomass (biofilms) can build a resistance to just about any anti-microbial initiative. As well, there are many cases where there is no residual impact of an anti-microbial treatment (such as with ultraviolet radiation or other non-chemical disinfection devices), and others still where it is not practical to measure the residual in the field (such as with numerous non-oxidizing biocides).

ORP (Oxidation/Reduction Potential)

Oxidation Reduction Potential (ORP, or Redox) is a measure of a water system's capacity to either release or gain electrons in chemical reactions. The process of oxidation involves losing electrons while reduction involves gaining electrons. Oxidation and reduction

(redox) reactions control the behavior of many chemical constituents in water. ORP values are used much like pH values to determine water quality.



While pH values characterize the relative state of a system for receiving or donating hydrogen ions (acting as a base or an acid), ORP values characterize the relative state of a system for gaining or losing electrons. ORP values are affected by all oxidizing and reducing agents, not just acids and bases.

The life expectancy of bacteria in water can be related to ORP. In fact, studies have shown that the life span of bacteria in water is more dependent on the ORP value than on the chlorine concentration. However, the ORP measurement by itself does not provide any indication of the biological population and therefore it does not provide sufficient basis for process control on its own. When used in conjunction with other process parameters, it can provide value.

Nutrient Measurements

The measurement of **Total Organic Carbon (TOC)**, **Chemical Oxygen Demand (COD)**, **Ammonia-Nitrogen (NH₄-N)**, **Dissolved Oxygen (DO)** and other nutrients required for microbiological growth are an excellent means to assess their potential for growth, but by themselves say little about specific microbiological activity. Even when measuring the removal of a nutrient, one must consider that most any biological nutrient can be consumed or transformed by chemical or physical means in addition to the biological option.

Once again, such measurements are strongly complementary to microbiological measurements, but alone do not speak to microbiological concentration, activity or quality.

Summary – What Does it All Mean?

Microorganisms are ubiquitous in the environment and by consequence in any municipal, commercial and industrial process. They pose real threats to product quality, process stability, equipment integrity, human health and in many cases, to the environment. The traditional tests for microorganisms have existed since the late 19th / early 20th century, but many new methodologies have expanded the diversity and relevance of the microbiological toolkit. When these methods are used as intended, they can empower experts and operators alike to make better and more rapid decisions on how processes can and should be controlled to mitigate these threats.

2nd Generation ATP monitoring is one of these revolutionary new tools, filling the specific need for a rapid and direct measure of total microorganisms. It is a test method that can and will serve this need for a long time to come and is **complementary** to the other options that are out there for quantifying and qualifying microorganisms!

The next page presents a tabular comparison of all the methods described in this document.

Method Comparison Table

Method	2 nd Generation ATP	Microscopic Examination	Culture Tests	Molecular Biology Methods	Particulate Analysis	Bioassays	Respirometry	Chemical Methods
What is detected?	Total Microorganisms	Total or Specific Microorganisms	Culturable Microorganisms	Specific Microorganisms	Suspended Solids	Toxicity to Surrogate Organisms	Metabolic Activity	Chemical Constituents
Interferences in detecting total living biomass	None	Dead biomass; non-biological particles	<i>UNABLE TO MEASURE</i>	<i>UNABLE TO MEASURE</i>	Dead biomass; non-biological particles	<i>UNABLE TO MEASURE</i>	Respiration Type	<i>UNABLE TO MEASURE</i>
How long to get results?	Minutes	Minutes to Hours	Days to Weeks	Minutes to Days	Minutes to Hours	Minutes to Days	Minutes to Hours	Minutes
Can give results on-site?	Yes	Yes (but difficult)	No	Yes (in some cases)	Yes	No	Yes	Yes
What types of samples can be tested?	Fluids & Solids	Fluids & Solids	Fluids, Re-suspended Solids	Fluids, Re-suspended Solids	Fluids only	Fluids, Re-suspended Solids	Fluids only	Fluids & Solids
How much skill is required?	Low	High	Moderate	Moderate to High	Low	High	Moderate	Low
What is the capital cost?	Moderate	High	Low	Moderate to High	Moderate	Moderate to High	Moderate to High	Low to Moderate
What is the cost per test?	Moderate	Low	Moderate	Moderate to High	Low to Moderate	High	Low	Low to Moderate
What is its best use?	Total microbiological concentration	Population diversity	Specific microbiological concentration	Population diversity & specific concentration	Total particles	Environmental toxicity	Specific metabolic activity	Chemical constituents