Real-Time Microbial Detection.....
The Future of Mold, Bacteria and Water Damage Testing

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- Background -

The indoor environmental quality (IEQ) industry is often described as being in constant dynamic change, modification and improvement. These characteristics enable industrial hygienists and related environmental scientists, microbial remediation/water damage contractors, product suppliers as well as insurance companies the unique ability to embrace and adequately utilize new technologies and methods for performing their work. A new technology being introduced into the IEQ field is the use of real-time microbial detection for testing the biological contamination level of building material surfaces. These building material surfaces may have been compromised due to water damage and incursion events, high humidity conditions, poor surface hygiene cleaning, etc. Real-time microbial detection is made possible by measuring the concentration of adenosine triphosphate (ATP) found within each living cell of the biological contamination that may be present, including fungi, bacteria, biofilms, somatic cells, etc. Sampling for the level of ATP on affected surfaces, is and has been for many years, a widely accepted scientific method of determining the level of bio-contamination by measuring the bioluminescent or light byproduct created by the biochemical reaction between luciferin/luciferase reagent and the ATP molecule within every viable organic cell. The advantages of this type of surface testing are results known within 30 seconds of sample collection, cost reduction of 10-30 times less than culture testing or microscopy analysis and the accuracy and sensitivity of ATP technology is excellent. In addition, ATP testing is easy for anyone to perform and interpret. Recent IEQ industry standards such as the IICRC S520 have allowed for adequate application and interpretation for new technologies such as ATP testing to become a recognized method of microbial and biological testing.

- Traditional Environmental Testing Methods -

The IEQ industry has a rooted history of using conventional culturing methodology for viable as well as non-viable sample collection to evaluate the hygienic level of the sampled environment. This approach inherently relies on the technical capabilities of a skilled technician to collect good samples and the use of a laboratory for analysis of those collected samples. These methods provide information about the number of microbes present on the sampled or contaminated surface and also have the advantage of being able to detect specific indicator organisms. Typically, biological (fungi and bacteria) surface sampling for culturable organisms consist of swabbing, wiping, micro-vac method, etc. and the analysis may require days to weeks to obtain results, which may cause delays in project completion or rebuild of remediated areas. Ultimately, these time delays in the project could lead to excessive costs related to re-occupancy of work areas, additional cleaning and decontamination if the sample results fail and additional testing fees associated with the project to reach final clearance acceptance. Additionally, these methods reveal little to nothing about the biological residue left on the surface that can support the survival and regrowth of microbes.

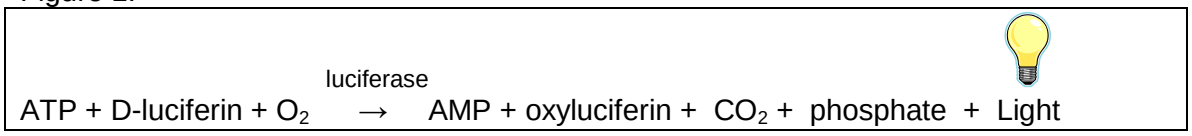
- The Ideal Objective – Cleaning and Testing –

The primary objective of any biological contamination remediation (fungi or bacteria) is to physically remove the biological debris or contamination from the substrate or surface. An ideal test to measure the efficacy of the biological contamination remediation and hygienic status is a test for overall biological residue itself. This method would allow for rapid results in an attempt to facilitate immediate corrective action and be simple enough to be performed by the Indoor Environmental Professional (IEP) or as a means to measure the effectiveness of work performance or quality assurance by the remediation contractor prior to the involvement of an independent party, such as an IEP. The ideal method and technology to accomplish the testing of the presence of the biological residue is measuring ATP bioluminescence of the biological contamination of the sampled surface.

- Bioluminescent ATP Assay Technology -

In the 1980's, the detection of ATP bioluminescence was applied to the detection of microbes in foods as well as measuring the hygiene status of process surfaces. Adenosine triphosphate (ATP) is the chemical compound found in all organic matter including fungi, bacteria, somatic cells, plant cells, etc. ATP is known biologically as the "universal energy carrier" within living cells and is a significant biochemical component of the Krebs Cycle. In the ATP-luminometric test, the firefly enzyme (luciferase) in the presence of its substrate, luciferin, oxygen and magnesium ions catalyzes conversion of chemical energy of ATP into light through oxidation-reduction reaction (Figure 1).

Figure 1:



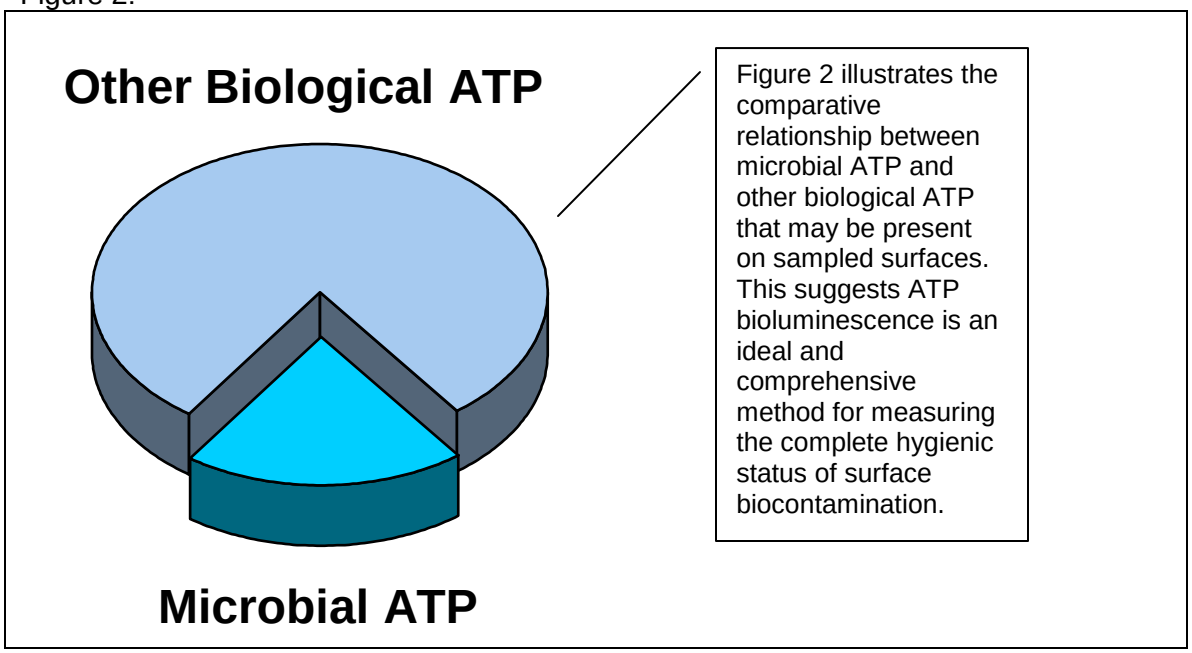
The quantity of light generated is directly proportional to the amount of biological ATP present, thus, the light units can be measured to estimate the biomass of cells in a sample. With state of the art equipment, and highly purified reagents, it is possible to detect trace amounts of microbial ATP corresponding to approximately $10^2 – 10^3$ in concentration. Quantification of intracellular microbial ATP can be conveniently accomplished using rapid and simplified extraction and assay procedures. The light emitted by this process can be monitored by a variety of luminometers. Supplying companies provide customers with test kits with all necessary reagents. The reagents are injected into the instruments and readout is reported in relative light units (RLUs). By knowing the number of microorganisms responsible for generating known RLUs, one can estimate the number of microorganisms in the collected sample. This correlation between surface cleanliness and microbial plate counts has made ATP bioluminescence a widely accepted method for the food, healthcare, industrial manufacturing and pharmaceutical industries.

This version of the ATP bioluminescence method based on detecting all ATP on a surface involves collecting samples by swabbing the surface. Reading of the bio-luminometers may be assessed numerically or as "acceptable" or "unacceptable". The procedure can be easily performed by almost anyone, with little training, in less than one minute. Portable luminometer reading units test swabs with pre-packaged reagents. The user swabs the surface to be tested, activates the swab by placing it into the

solution of reagents then inserts it into the chamber of the luminometer to obtain the measurement.

Additionally, significant interest has been generated in using ATP estimation not only for total viable cell counts but as a surface hygiene check including the verification of non-culturable cell presence on a surface, which allows the user of ATP bioluminescence technology to evaluate the total bio-burden of the contaminated surface and not just culturable fungi or bacteria (Figure 2).

Figure 2:



- Using ATP Bioluminescence and the IICRC S520 -
- Methodology -

The purpose of using ATP bioluminescence to evaluate the biological conditions of non-water damaged, water damaged and microbial contaminated building materials is to provide some numerical value of biological contamination for interpretation of Condition 1, 2, and 3 surfaces as outlined by the IICRC S520 Standard. In order to perform this evaluation, significant quantities of ATP surface swab sampling were performed in the field at a variety of project sites and of various surface types (unpainted wood framing, unpainted plywood sheathing, unpainted oriented strand board sheathing, painted and unpainted gypsum board materials, horizontal hard surface table tops, hard surface flooring and sheetmetal surfaces) using an ATP bioluminescence testing system. The materials sampled in the field were all categorized into one of the three conditions consistent with the IICRC S520 definitions based on visual observations and / or proximity of the sampled surfaces to visual water damage or fungal growth of other surfaces. Moisture content testing of the sampled material was performed with the use of a moisture meter to document the general condition of the sampled material at the time of sampling. The sampling area was consistently two inches by two inches square and the ATP swab collection device was used by applying ten strokes in either direction while rotating the swab in an attempt to collect the most consistent sample. All of the ATP swab samples were analyzed in a luminometer and the results were recorded on-site.

- Summary of Findings –

The results of the ongoing field evaluations using ATP bioluminescence testing as outlined in the methodology section above yielded the following summary of findings.

Surfaces sampled were relatively dry or had been impacted with water and were near dry when sampled for ATP.

Sampled Surface Condition	Moisture Content %	ATP Testing Results (RLU)	IICRC S520 Condition
No visible microbial growth	<15%	1 – 150* (typ. 1 – 75)	1
No visible microbial growth – within areas with visible microbial growth	<15%	50 - 150	2
Visible microbial growth	<15%	>150	3

* - ATP results relative to Condition 1 were dependent on the overall housekeeping cleanliness within the sampled location or on the sampled surface. Typical Condition 1 surfaces yielded ATP results that ranged from 1 RLU to 75 RLUs; however, there were samples that were as high as 150 RLUs.

Surfaces sampled were relatively damp or contained active moisture incursion with water when sampled for ATP.

Sampled Surface Condition	Moisture Content %	ATP Testing Results (RLU)	IICRC S520 Condition
No visible microbial growth	< 15%	1 – 150* (typ. 1 – 75)	1
No visible microbial growth – within areas with visible microbial growth	< 15%	100 - 500	2
Visible microbial growth	≥ 15%	>500	3

* - ATP results relative to Condition 1 were dependent on the overall housekeeping cleanliness within the sampled location or on the sampled surface. Typical Condition 1 surfaces yielded ATP results that ranged from 1 RLU to 75 RLUs; however, there were samples that were as high as 150 RLUs.

- Conclusions -

Based on the results of the ATP bioluminescence field testing in a variety of conditions and of various material types, the following conclusions can be made.

- The type of materials sampled for biological contamination (unpainted wood framing, unpainted plywood sheathing, unpainted oriented strand board sheathing, painted and unpainted gypsum board materials, horizontal hard surface table tops, hard surface flooring and sheetmetal surfaces) did not appear to have any significant physical limitations that would negatively impact the collection of consistent surface swab samples.

- The level of moisture content of the sampled materials did have significant impact on the total level of ATP present in the sample with respect to Condition 3 situations. The higher the moisture content (>15%) of the material, the higher the

ATP levels detected. This is due to the biological growth activity of the microbial surface contamination in the presence of elevated water activity. Although there are very small amounts of ATP in fungal spores, the largest amount of detectable ATP is found in the hyphae and mycelium of fungal growth contamination. This does allow for some relative measure of the overall viability or health of fungal growth as well.

- The ATP level of Condition 1 situations was significantly dependent on the overall level of hygiene within the sampled space and of the sampled surfaces. A majority of the Condition 1 situations yielded results that ranged from 1 RLU to 75 RLUs; however, there were instances where ATP levels were measured as high as 150 RLUs. Biological debris accumulation can lead to increased levels of ambient ATP on unaffected surfaces or surfaces not within Condition 2 or 3 situations. However, this condition of unhygienic surface contamination provides valuable insight into the level of potential remediation and cleaning that may be required to improve the overall quality of the indoor environment.

- The ATP level of Condition 2 situations was slightly increased in the presence of Condition 3 surfaces with moisture contents that exceeded 15%. This is suspected to be due to the increased viability of the fungal contamination in Condition 3 situations that may be impacting or creating Condition 2 situations. The biological debris accumulation in these Condition 2 situations appears to consist of more viable fungal matter.

- Significant evidence exists that supports the use of ATP bioluminescence as a method of analyzing the biological contamination levels (fungi, bacteria, somatic cells, etc.) of surfaces. The consistency and efficiency of the ATP biochemical reaction that occurs improves accuracy and consistency of the sampling results and is effective in allowing the user to make “on the spot” determinations relative to Conditions 1, 2 and 3 as outlined in the IICRC S520 standard. However, applying field experience to specific on-site conditions as well as understanding that ATP testing includes all viable biological contamination and not just fungi, will improve the interpretation of the data collected. This also makes ATP testing a good choice for all types of field evaluations relative to biological contamination, including fungal contamination projects, sewer backup projects, general water infiltration evaluations and general assessments of indoor environmental hygiene in home, commercial, educational and healthcare settings.

- The use of ATP bioluminescence allows for fast (30 seconds to one minute) determinations in the field as to the overall biological contamination of various impacted surfaces. The relative inexpensive cost of each sample ($2 - $3) combined with improved portability make ATP bioluminescent testing a fast, reliable, cost effective and relatively accurate method of determining surface biological contamination.

References

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