A Look at COVID 19 in Terms of: 9. Using ATP & the Mycometer® for Assessing Cleaning Completeness

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Introduction

This is a continuation of a series of papers on COVID 19 with respect to health & safety. It focuses on two specific testing methods that might be used to evaluate the efficacy of cleaning processes for COVID 19. Like the other papers (most in preparation), this paper is intended to provide the facts and research support where available, and then draw from that as well as the author's experience to provide recommendations based on the weighting of the evidence. The data provided here, and the recommendations, cannot be maintained in a vacuum, thus other COVID 19 topical papers are intended to follow this one, but each will hopefully be sufficiently self-contained to be useful and reliable. The intended audience of this paper is professionals. This includes industrial hygienists, occupational and public health professionals, health and safety practitioners, and medical personnel. It is not intended for the general public, though many individuals may benefit from reading it.

ATP Reaction for Testing

Adenosine Triphosphate (ATP) is an energy carrying molecule (see Figure 1) in all living cells[1] and can as such be taken as indirect measure for cell density. Viruses do not store ATP and thus are not able to be directly evaluated by ATP tests (It is interesting to note that they do have protein motors that act as ATP enzymes[(4)]). ATP for purposes here is measured as a bioassay, where a luciferase enzyme (from firefly *Photinus pyralis*) catalyzes an oxidation by O2 of D-luceferin using ATP as energy source. Thus, reducing Adenosine Triphosphate to Adenosine Diphosphate and releasing the freed energy as light.

[1] **There are many interesting debates over whether viruses are living** (see for instance 1 **Forterre, P.:** To be or not to be alive: How recent discoveries challenge the traditional definitions of viruses and life. *Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences* 59: 100-108 (2016), 2 **Brüssow, H.:** The not so universal tree of life or the place of viruses in the living world. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364(1527): 2263-2274 (2009), 3 **Villarreal, L.P.:** Are viruses alive? *Scientific American* 291(6): 100-105 (2004).). **For my purposes, they do not produce ATP and will be placed in the non-living category for convenience.**

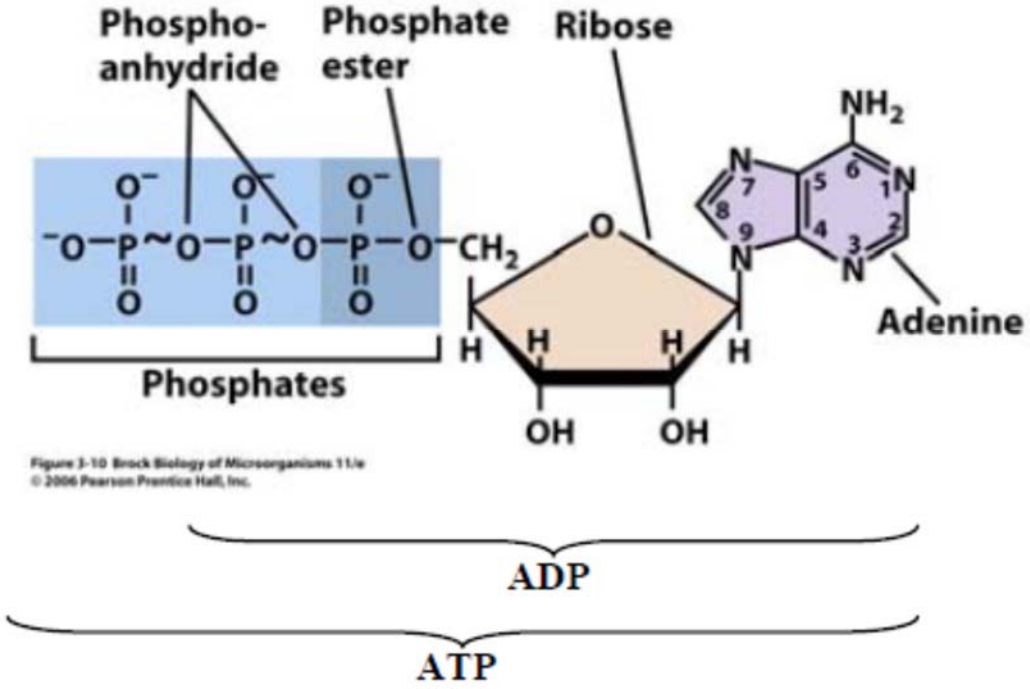

Figure 1. Molecular structure of Adenosine Triphosphate (ATP) (after Cofitzen, 2006)

The light from this reaction (see Figure 2) is measured in a luminometer, typically as Relative Light Units (RLU) and converted to ATP values by a calibration curve based on ATP standard salt. A number of commercial luminometers and reagent-kits exist on the marked. These can vary by application, but all include the following steps: a) addition of an extraction reagent to the sample, b) an extraction period, c) addition of the luciferase/luciferin reagents, d) an integration period. The measurement can be usually be performed in minutes.

$$ATP + D-luciferin + O_2 \xrightarrow[+\,Mg^{2+}]{firefly\ luciferase} AMP + PP_i + oxyluciferin + CO_2 + light$$

Figure 2. ATP luminecence reaction (after Cofitzen, 2006).

The ATP value cannot be converted directly to number of bacteria or fungi, since the ATP content of the single cell depends upon bacteria/fungi type and its growth phase. However, within a specific environment, pragmatic correlations can be drawn.

Mycometer® for Bacteria

For the Mycometer®, two methods are used, one for bacteria and one for fungi. In the BactiQuant™ for bacteria method a bacterial enzyme hydrolase (BH) is used as indirect measure of cell density. A substrate containing the fluorescent compound 4-Methyl Umbelliferone (MU) (See Figure 3) binds with the specific enzyme, thereby releasing the MU-ion, which then can be detected in a fluorometer. The result is provided as relative fluorescence units (FE).

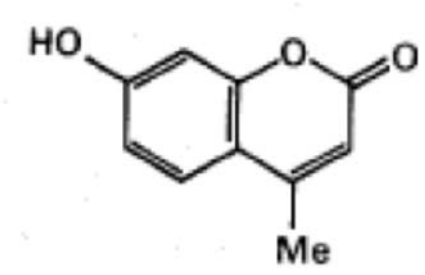

Figure 3. Molecular structure of fluorescent 4-methyl umbelliferone used in the BactiQuantTM method (after Corfitzen, 2006)

As with the ATP method the result cannot be directly converted to a bacteria count, as the content of the specific enzyme depends on bacteria strain and its growth stages. It must be correlated under more specific conditions/applications.

Mycometer® for Fungi

The Mycometer®Surface for mold method use a fluorescent substrate, 4-methylumbelliferyl N-acetyl-b-D-glucosaminide (MU-NAG) (See Figure 4) to assess the enzymatic activity of N-acetylhexosaminidase (NAHA) (See Figure 5). The fluorescent compound (MU) release can be detected in a fluorometer. The result is provided as relative fluorescence units (FE).

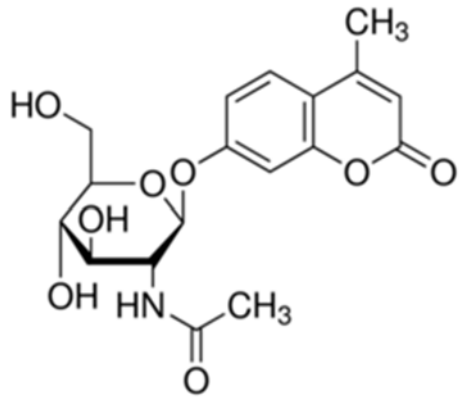

Figure 4. The fluorescent compound 4-methylumbelliferyl N-acetyl-b-D-glucosaminide (MU-NAG) used in fungal detection.

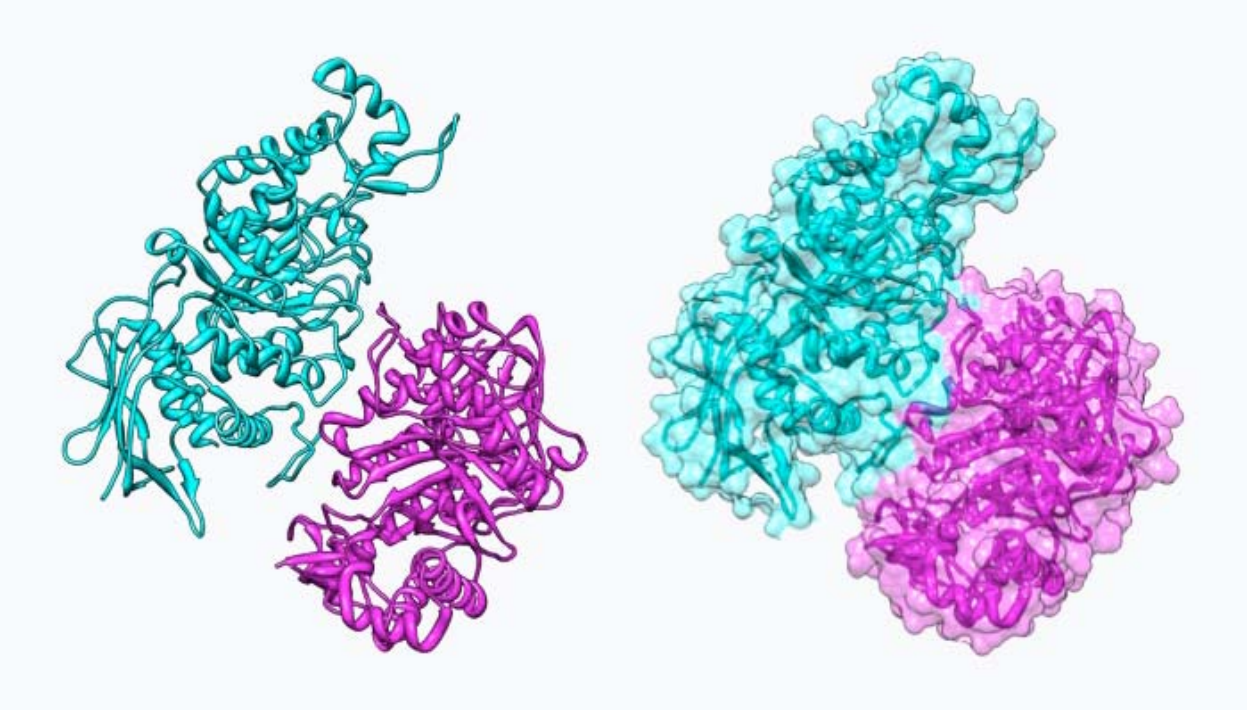

Figure 5. N-acetylhexosaminidase [Hexosaminidase A] (NAHA) used to assess fungal mass (Source: Wikipedia].

As with the ATP method and the Mycometer® bacteria method, the fungal result cannot be directly converted to a fungal count, as the content of the specific enzyme depends on fungal taxa and its growth stages. It must be correlated under more specific conditions/applications. However, significant correlations between NAHA and total spore counts were found in air samples and in dust generated from biomass in a biofuel plant[5, 6]. Strong correlations have also been found between fungal biomass (gravimetric weight) and NAHA in fungal species grown on nutrient agar and between ergosterol and NAHA activity on mold contaminated gypsum boards[7]. A linear correlation between NAHA and *Aspergillus niger* biomass has also been reported[8]. These studies demonstrate that NAHA can be used as a marker for mold cell biomass under the proper conditions.

ATP Application

ATP testing by luminous reaction as a hygienic tool has been used in the food & beverage industry[9-12], medical facilities[13-17], offices[18], industrial water treatment plants[19, 20], manufacturing[21, 22] and schools[23, 24]. ATP has been used to evaluate bacteria[9-11, 13-17, 19-22, 24], mold[7, 9, 12, 21, 25], and even viruses[18] under a surrogate basis. In one study[21] it was found to have a lower detection limit than culturing of bacteria and fungi.

Mycometer® Application

Using specific cellular components and a fluorescent response, the Mycometer® has been used to assess bacteria[19, 20, 23, 26-30] (via and fluorescence), and mold[7, 26, 28, 31-36] (beta-N-acetylhexosaminidase and fluorescence). Similar to ATP it has been used in food industry[30], commercial office space[25, 29, 31, 33], industrial water treatment plants[19, 20, 27], animal care facilities[32], and homes[29, 31, 33-35]. The manufacture has recommended increased surface area for swab collection and using a longer reaction

time along with a lowered acceptable limit (below detection) for those wanting to apply it to COVID 19[37]. This has reported resulted in a 10-fold increase in sensitivity.

Application for COVID 19

As noted above, a few things are important caveats in the application for ATP and the Mycometer® to determining level of cleanliness for a COVID 19 cleaning evaluation. The first is that neither ATP nor the Mycometer® directly determine viral loading in an airborne environment or on a surface. They can, each in their own way, measure a mixture of biological material that indicate human cellular material (ATP only), along with that from a variety of bacteria and fungi[24](both methods). Such material includes epithelium from upper respiratory mucus membranes (mouth, throat, nasal passages) from saliva and exudates and associated material from coughs and sneezes from persons with viral as well as bacterial infections. Because viruses are associated with living cells (viruses need them to replicate) ATP is an overall generic marker of biological contamination, and it allows one to monitor potential viral contamination (from viral infections) indirectly[24]. One study with M-2 phage (virus)[18] found this surrogate approach (ATP) successful. Similarly, the Mycometer provides a way of monitoring biological load. In general, The Mycometer® is more specific and ATP is broader in the scope of biologic indicators being measured. Because bacteria are organisms more likely to correlate with the presence of human shed viruses than mold would be, the use of the Mycometer® bacteria method is more appropriate than the fungal method for assessing COVID 19 cleaning processes.

Cautionary notes are in order. These methods assess biological load, this can be both alive and dead for ATP; it is mostly alive for bacteria by the Mycometer® and a combination of both alive and dead for mold by the Mycometer®[37]. Using a disinfectant application method that is intended to kill these organisms “on contact” does not remove them from the surface nor does it alter the chemical/molecular structure sufficiently to eliminate them or their constituent parts. One should expect to find residue of these organisms if they are not physically removed from a surface. If one intends to use ATP or the Mycometer® for assessment of cleaning, one should require physical cleaning. One should also be aware that many biological sources have ATP, e.g., wood, plants, insects, starch from gloves, etc. Many substances have natural fluorescence: certain living organisms, minerals, and even polymers. Thus, one should recognize the potential for false positives depending on the surface contamination aka dirt and debris).

These methods are best applied to qualitatively assess performance of cleaning, i.e., don’t use them to show that viruses are present. Nor should one state that a pre-cleaning finding indicates a known risk. These tests may, if properly structured, be able to assess relative cleaning effectiveness (as opposed to absolute), i.e., pre:post effects. These methods are correlated by providers of the kits or instruments for certain types of applications, certain areas of testing, and certain temperatures and environmental conditions. Not adhering to these correlating conditions will likely result in unusable data. One sample is not a statistic. One needs to collect a sufficient number of samples to properly assess the exposure and/or risk. The author has found that somewhere between 5 and 20 samples from a homogeneously contaminated and cleaned area often provides sufficient statistical power to make informed decisions[38]. Users of these tests should provide adequate support for their choice of number of samples prior to sampling.

In conclusion, both ATP and the Mycometer® Bactiquant® testing can be used to assess COVID 19 cleaning process completeness in a surrogate fashion, if applied properly and consistently. These tests

take less than an hour to perform in the field. The alternative is to conduct sampling of surfaces with swabs and have them shipped to a lab and analyzed for particular RNA patterns by PCR that will definitively identify Sars-CoV-2[2] (COVID 19) to 5 viral genome units per reaction – but the wait is 5-7 days.

Disclaimers

The author owns a Mycometer® and has used it for evaluating mold and bacteria (including MRSA), performed trials on fogging and surface cleaning, and has performed work with ATP meters. The above does not constitute an endorsement for either instrument type, but rather a presentation of the data available to the author. The author has not received payment, commission, or preferential service for writing this paper. The author did contact a Mycometer® representative prior to completion of this paper to verify certain claims made by others about their equipment. Users should educate themselves on their instruments, the assumptions and limitations of the equipment, the environment for sampling, and then manage the risk appropriately.

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