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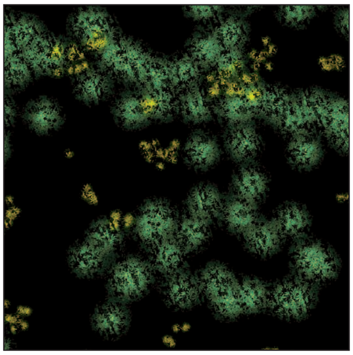
ATP Testing Systems for Measuring Cleanliness



Since my first day as a sanitarian, I have been vexed by the question, "How clean is clean?" Throughout my career the answer to this question has been punctuated by differences of opinion among my public health inspection colleagues, particularly in evaluating food contact surfaces and citing them for not being clean to "sight and touch." I've always viewed this standard as an aesthetic opinion: What is deemed clean to one individual may not be considered clean by another. If the point of making public health inspections is to reduce the contamination—or cross-contamination—levels of people, objects and surfaces in the food production environment, is it not our goal to ensure bacterial cleanliness?

The differences between bacterial and aesthetic cleanliness lie at the opposite ends of the spectrum in terms of their relevance to diseases pertinent to food safety. In the world of environmental microbiology, cleanliness is defined in several ways, usually by degree of microbial removal. The highest standard of cleanliness is "sterility required," where the maximum allowable number of organisms is somewhere around 10^6 per unit volume or area. This is followed by the "removal of pathogens, lowest possible level of other microorganisms," which is the definition of disinfection, sans reference to sporeformers. Lower on the scale of microbial cleanliness is the reduction of microbial numbers to levels considered "safe," whatever that means. In public health parlance, this is commonly known as sanitization or antisepsis when applied to people and other living things.

At this point, cleanliness further finds its expression by defining a descending order of clean. Aesthetic cleanliness, when applied to food safety, refers to the hygiene of utensils and food contact surfaces and is measured by observing surfaces that glisten and are squeaky-dry to the touch. Keeping unwanted microbes from growing is a lower priority and refers to the cleanliness of "floors, walls and ceilings," a standard that is still a major portion of most regulatory inspections. Finally, the last priority in the how-clean-is-clean hierarchy is preventing foul odors, such as in and around the kitchen drains and waste disposal systems. Oh, the bane of subjectivity.

Obviously, to arrive at a more precise definition of clean requires objectivity. Under ideal conditions, with adequate time and resources, we can measure the bioburden of any processing area, food equipment or utensil with an arsenal of swabs and sponges complemented by nutrient broths and agars. Unfortunately, as regulators, auditors and quality control professionals we often do not have that luxury in time or materials. To complicate matters, the FDA Food Code has given us a new paradigm of risk, with a target of hazard analysis. Therefore, not all surfaces, processes, and conditions are evaluated equally, or indeed, need to be. The question then is: How can we objectively measure degrees of microbiological cleanliness in real time? The short answer is we can't. But we can easily and economically measure the cleaning processes and subsequent efficacy of biological cleanliness. Mind you, we're using the term biological rather than microbiological cleanliness, but more on this later. Enter ATP technology.

A Bit of History

Although adenosine triphosphate (ATP) was first discovered in 1929, it wasn't until the late 1980s and 1990s, along with the refinement of Green Fluorescent Proteins (GFP) and subsequently luciferin/luciferase, that ATP testing was developed. The ATP testing technology used to measure biological cleanliness in our industry is in its relative infancy. I first learned of it in 2000 and got my first ATP test kit a year later. It was a bulky and weighty affair that worked rather well with a bit of training. However, it was neither easy to operate by today's standards, nor entirely "portable" for routine use in the field. The primary limitations were the labile nature of the reagents and complexity of the kit and analysis. Since then, the refinements in both the instrumentation and technology of the test have been no less than remarkable. The price for these units has come down as well. For these reasons, ATP testing is rapidly becoming a standard in our industry, albeit with a host of misconceptions of its utility and misinterpretations of data. For this reason, I would like to briefly introduce you to the science and data interpretation, as well as a few suggestions on the selection of instrumentation that will enhance quality control, quality improvement and quality assurance programs.

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The Science and Technology

ATP is the primary energy transfer molecule present in all living biological cells on Earth. ATP cannot be produced or maintained by anything but a living organism, and as such, its measurement is a direct indication of biological activity. Because the level is strictly-controlled in a living cell, ATP determination is used as an indicator of viable cell numbers. For hygiene testing the total ATP content of the sample is determined. This includes both eucaryotic and microbial ATP. The purpose of ATP testing is to achieve and defensibly document effective cleaning by following the principle that if biomass is not extant on critical surfaces after cleanup there is not enough medium for microbiological proliferation. Simply stated: no biological contamination, no microbial growth.

The main advantage of ATP as a biological indicator is the speed of the analysis. Unlike quantitative microbiological monitoring that requires at least several hours, quantitative biological monitoring takes only minutes from collecting the samples to obtaining the results. Results are given in real time. Here is how it works: ATP is rapidly detected by light emission through the combined use of luciferase and a luminometer. An ATP free swab is moistened with an ATP free buffer, water or extractant. The use of the extractant helps releasing ATP from the surface being sampled. Using a portable luminometer, testing the swab is usually done immediately. There are some systems where the swabs are stable for a number of hours; thereby allowing the user to complete the analysis at a workstation or laboratory.

There is no correlation between ATP and standard microbiological methods such as total plate count on hard surfaces, or in any materials contaminated with other organic matter. Therefore, for our purpose, totaling the ATP readings (microbial + residue) found on a surface is the most common and reasonable approach recommended for hygiene monitoring. Correlation of the ATP readings with CFUs has been attempted by various researchers with varying successes–or failures. Determining the ATP level for microbes is possible.

However, a strong word of caution: Since both viable and non-viable microbial ATP is measured, a selective extraction methodology is used to separate the two. First, non-microbial ATP is extracted with a non-ionic detergent (generally, Triton X-100) and then destroyed by treating with a high level of potato ATPase for 5 minutes. Subsequently the microbial ATP is extracted using a 5% solution of trichloroacetic acid, and an organic solvent such as ethanol, acetone or chloroform. This requires subsequent dilution to avoid luciferase inhibition. Since the level of ATP in eucaryotic cells is three orders of magnitude greater than bacterial cells, this procedure is difficult to achieve and its reliability is questionable. Therefore, for all intents and purposes, ATP testing is not a substitute for plate counts and pathogen testing, even though the temptation is there to use it as such in this age of expediency.

Interpretation of Results

There is a grave misunderstanding in our industry that the ATP monitoring system is a "bug test" when it really is a "dirt test." I would be remiss if I did not advise you about some of the nuances of data interpretation. The test readout is in relative light units (RLUs), which mean the systems measure luminescent units, not cells. Remember, the luminometer detects bioluminescence from ATP, a cell constituent. In searching the literature, I was unable to find any regulatory limits on RLUs, only several admonishments to set one's own limits depending on operations monitored and use of the kit.

It is generally accepted that clean surfaces show low levels of total ATP. On the low end of sensitivity, most test kits will detect less than 0.5 picograms of ATP from bacteria, somatic cells, or both. For comparison use only, this number is equivalent to about 1,000 bacterial cells. It is therefore safe to assume that light output (in the luminometer) greater than two to three times background of a clean surface indicates that the area tested is contaminated with biological material. However, the method is very sensitive, and in practice, a threshold of 10 times the background readings can be accepted in quality control analyses. Nevertheless, some preliminary work is required to establish the relevant pass/fail limits for the test. This is best accomplished by collecting reference data in accordance with a recognized sampling plan such as ANSI/ASQ Z1.4, following normal cleaning procedures. The level set will depend on the type and condition of the surface and the cleaning methods used.

Considerations When Choosing an ATP System

I would like to offer a few suggestions about how to select an ATP testing system. By considering all of these factors carefully, foodservice and food processing facilities will find the system that provides good scientific-based indication that food contact surfaces are indeed clean and offers cost-efficiencies to the business.

- Consider the instrument's precision of results from a percentage standpoint when using a consistent amount of ATP. All instruments have different scales; the variability of results is given as a percentage of the mean value of the data set. In other words, the instrument should be accurate across its entire scale of detection. Likewise, ease of calibration or validation and reproducibility of results are also important factors in instrument selection.
- Consider the testing environment. Specifically, how well will the swab chemistry hold up to sanitizers and other chemicals in the production and plant environment, as well as to variations in temperature. This is particularly important if the unit is taken into the field under different ambient environmental conditions. Utility and ruggedness count.
- Consider the shelf life of the swabs and the recommended conditions for their storage (room temperature versus refrigeration). This is important if the system is truly portable and will be taken from site to site.
- Ease of swab and test use can be important when time, movement and test conditions such as safety and prevention of contamination are considered. For instance, if ATP testing involves going on ladders, narrow cat-walks, or over or into the interior of processing equipment, the simpler the test preparation, the better.
- Consider the availability and cost of replacement materials. Through anecdotal experience I've learned that "generic" swabs or swabs from another manufacturer other than the vendor of the kit, may not work as well. ATP systems, including the reagent chemistry, are designed to work as a unit, particularly as it relates to reproducibility of results in the presence of plant chemicals such as sanitizers. Therefore, considering the proprietary nature of the ATP test kit, consistency and availability of supplies are vital to the seamless and continued operation and use of the equipment.
- Finally, an ATP testing kit is only as good as the operator using it. The operator using a test kit is only as good as the support from the manufacturer or vendor selling it. Without appropriate training, educational and technical support, investing in an ATP system can be frustrating and nonproductive. It goes without saying that this may be the most important selling point when considering a unit.

"An ATP testing kit is only **as good as** *the* **operator** *using it."*

Unlike much of the field instrumentation used in food safety, ATP test kits are relatively new and are evolving as rapidly as technology allows. I've learned that we all made mistakes in the selection, use and data interpretation of the systems we selected. Find out from others what works and what doesn't. And, most importantly, try out a unit before buying, learn about its limitations and capabilities, refine your data analysis and educate, educate, educate.

An Educational Tool

The ATP instrumentation and kits that are available today are excellent teaching tools since they measure cleaning processes in real-time. Likewise, they are a powerful enforcement tool since they can maintain data within the unit for later analysis, but only when used as a pass/fail screening test. Most ATP units have some type of data capture software that is compatible with most personal computers. As an added bonus, some units also come with a combination or combinations of pH, temperature and conductivity probes that complement most audits by further helping describe the immediate environment of the test.

The more I use ATP testing in my work and the more I explain its operational capabilities and limitations to my clients, the greater is our collective level of comfort in defining and setting reasonable standards for cleanliness in food handling establishments and manufacturing plants. Ultimately, with the real-time data offered by ATP environmental monitoring, the question of "How clean is clean?" has become a little less vexing.

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Dr. Powitz welcomes reader questions and queries for discussion in upcoming columns, and feedback or suggestions for topics you'd like to see covered can be sent to him directly at sanitarian@juno.com or through his website at www.sanitarian.com.