
11:20
Outer Distal End
Test Point Passed:
50 RLUs
Return
3M

# Cleaning Monitoring Using Adenosine Triphosphate: Facts & Fiction

BY CRAIG WALLACE, BS, PRESIDENT—WALLACE STERILIZATION CONSULTING LLC

This series of self-study lessons on CS topics was developed by the International Association of Healthcare Central Service Materiel Management (IAHCSMM). Purdue University's Extended Campus and IAHCSMM both offer grading opportunities.

**Earn Continuing Education Credits:**

**Online:** Visit www.iahcsmm.org for online grading.

**By mail:** For written grading of individual lessons, send completed quiz and $15 to:
Purdue University - Online Learning
Young Hall, Room 527
155 S. Grant Street
West Lafayette, IN 47907

**Scoring:** Each online quiz with a passing score is worth 2 contact hours toward your CRCST recertification (12 hours).

**Subscription Series:** Purdue Extended Campus offers an annual mail-in or online self-study lesson subscription for $75 (six specific lessons worth 2 points each toward CRCST recertification of 12 hours). Call 800.830.0269 for details.

**For More Information:**
IAHCSMM provides online grading service for any of the Lesson Plan varieties. **Purdue University provides grading services ONLY for CRCST and CIS lessons.** Direct any questions about online grading to IAHCSMM at 312.440.0078. Questions about written grading are answered by Purdue University at 800.830.0269.

**LEARNING OBJECTIVES**
1. Review the fundamentals of organic soils and cleaning
2. Examine how adenosine triphosphate cleaning monitoring technology works
3. Compare adenosine triphosphate to other cleaning monitoring technologies

It is well documented that cleaning is a critical element of any infection prevention program. Proper cleaning of environmental surfaces in patient rooms or treatment areas can play a direct role in reducing the transmission of pathogens, and thorough cleaning of medical and surgical devices is critical to the effective disinfection or sterilization of those devices.[1] Cleaning is often a manual process that can be subject to significant variations in quality. Infection prevention quality control programs rely on rigorous testing of cleaning processes to ensure that cleaning has been effective. This lesson reviews the options for testing cleanliness and digs deeper into one of the available tools: adenosine triphosphate (ATP) technology.

## Objective 1: Review the fundamentals of organic soils and cleaning

The soils that contaminate room surfaces and medical and surgical devices will vary significantly in composition, depending on the specific source of the soil. Most soils of concern encountered in the healthcare environment will be human in origin. Blood, mucous, urine, feces, tissues and other bodily fluids and secretions end up on environmental surfaces, medical equipment and surgical devices. These organic soils are made up of combinations of biological compounds, which include carbohydrates, lipids, proteins, nucleic acids, and high-energy compounds (See Figure 1). The primary risk presented by residual organic soils is that the soil may contain and protect viable pathogens embedded within the soil itself. These microorganisms present a direct risk of infection to another patient or a healthcare worker.

Cleaning, by definition, is "the removal of contaminants to the extent necessary for further processing or for intended use."[2] The key phrase in this definition is "removal of contaminants." The objective of the cleaning process is the physical removal of soil. Cleaning is *not* a process intended to inactivate or kill microorganisms. This distinction is important, especially when we consider

**Components Found in Clinical Soil: Organic Compounds**

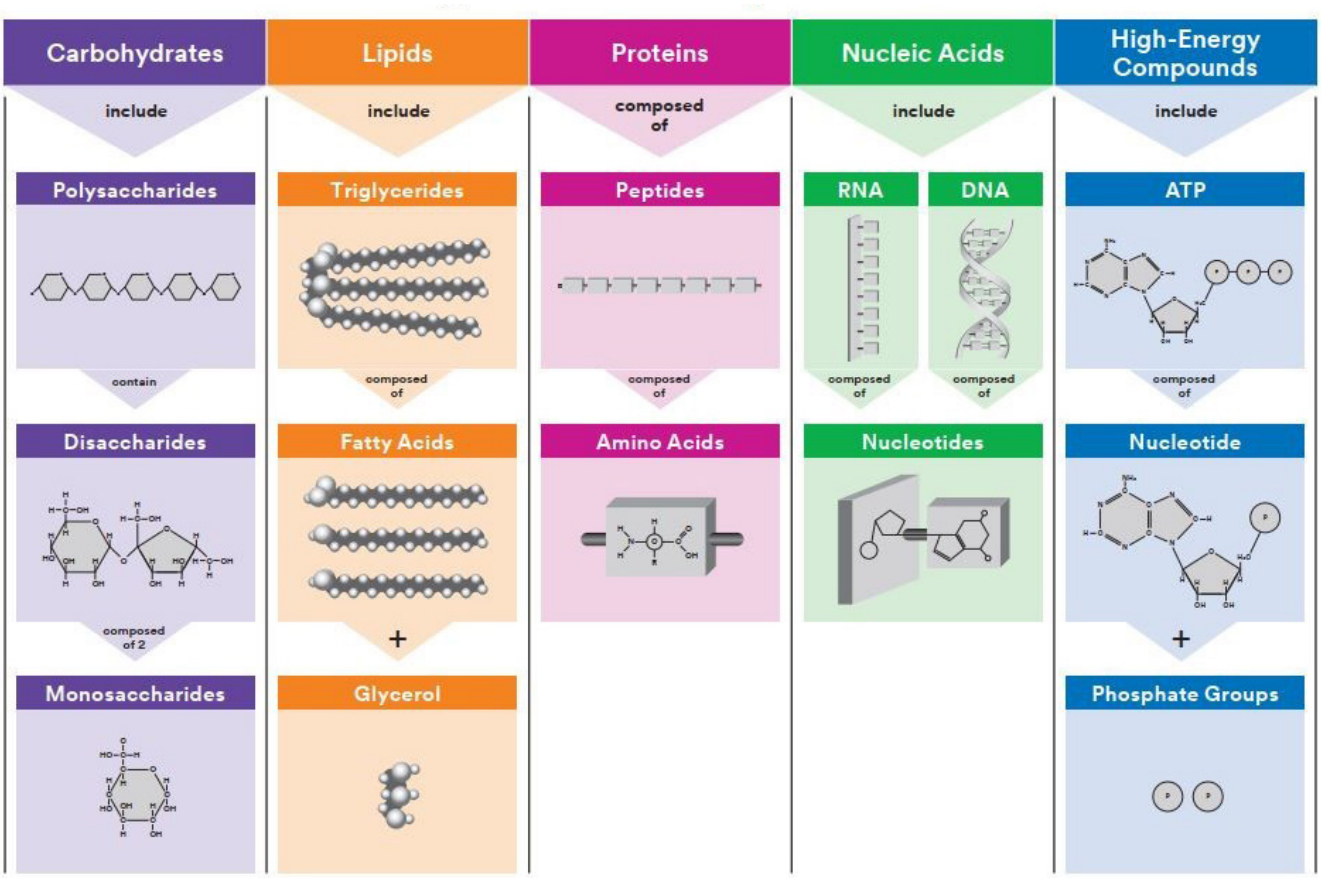


**Figure 1 – Composition of Organic Soils.** *Figure reprinted with permission from 3M*

how to appropriately test for cleanliness (more on this later in this lesson). A typical cleaning process will utilize a device that is designed to wipe, scrape or rub the soil away from the surface and allow it to be flushed away with water. These devices include brushes, sponges and wipes. Water under pressure and ultrasonic waves can also be used to physically separate the soil from the surface of a surgical instrument. Detergents and cleaners are used to chemically alter the soil, so it is more easily removed. Detergents use chemical compounds—called surfactants—that help dissolve the soil, so it is more miscible with water and more readily flushed away. Enzymatic cleaners use biochemical catalysts called enzymes to cut large soil molecules into smaller chemical pieces that are more easily removed.

The second part of the cleaning definition says cleaning is to be "to the extent necessary for further processing or for intended use."[2] Soil must be removed from medical and surgical instruments that are to be disinfected or sterilized before use. Any residual soil that remains after the cleaning process can act as a physical barrier and protect embedded microorganisms from contact with the disinfectant or sterilant, rendering the disinfection or sterilization process ineffective. Inadequate cleaning of environmental surfaces may result in viable pathogens remaining on a surface where they could be transferred to a patient or healthcare provider. In addition, inadequate cleaning that leaves organic soil with embedded microorganisms in place may lead to the formation of biofilm. Biofilm is layered bacteria encased in a polysaccharide matrix that is extremely difficult to remove from the surface and provides significant physical and chemical protection for the encased microorganisms.

For medical and surgical instruments, it is important to understand the difference between the cleaning process

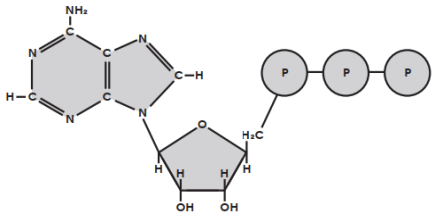

**Figure 2 – ATP Molecule**
*Figure reprinted with permission from 3M*

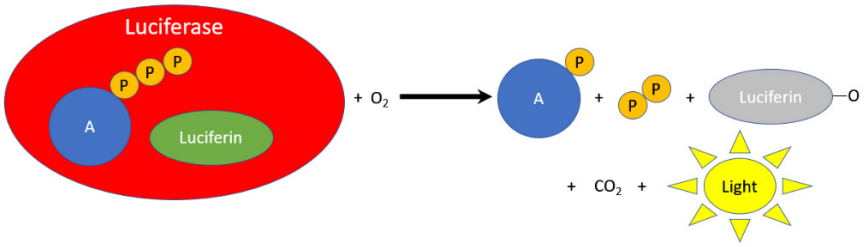


**Figure 3 – Luciferin/Luciferase Reaction with ATP**
*Figure reprinted with permission from 3M*

and the disinfection or sterilization processes. As previously stated, the purpose of the cleaning process is to remove soil from the device, so the subsequent disinfection or sterilization process will be effective. The purpose of the disinfection or sterilization process is to kill the viable microorganisms remaining on the device after cleaning. While the cleaning process will likely wash away some of the microorganisms embedded in the soil, it is not intended to kill microorganisms; therefore, the success of a cleaning process should be determined by measuring the amount of soil remaining after the process, not by measuring the presence of viable microorganisms.

Given the impact of cleaning on the safety of the surface or instrument, it is clear that the effectiveness of the cleaning process is critical to the effectiveness of the overall infection prevention program. Therefore, a quality control plan should be established for the cleaning process. This plan should include quality testing to provide information on the cleanliness of the surface or surgical instrument after cleaning has been completed. But how is this best accomplished? The original quality control test for cleaning was visual inspection (i.e., does the surface or item look clean?). Visual inspection of instruments may include added lighting or magnification[3] or, for endoscopes, a specialized lumen inspection device known as a borescope.[4] While still a required and useful first step, visual inspection is subjective and may not be able to detect small amounts of residual soil that could still be harboring embedded microorganisms. For this reason, an objective quality control test for cleaning should be able to detect and even measure very small amounts of soil that cannot be seen during visual inspection.

Again, organic soils can be comprised of many different types of biological compounds, and they vary by the specific source of the soil. If this is the case, how do we test for cleanliness? The accepted principle of cleaning monitoring is to test for certain specific types of chemical compounds that will be present in biological soil. These selected chemical compounds are called cleaning markers. Biological compounds used as cleaning markers include:

- ATP;
- Protein;
- Carbohydrates; and
- Hemoglobin.

The most common cleaning markers are ATP and protein as these compounds are present in the broadest range of biological soils.

## Objective 2: Examine how ATP cleaning monitoring technology works

ATP is categorized as a high-energy compound as it is used by living cells to store energy for later use, like a battery. It is used as a cleaning marker because it is present in the cells of all living organisms—and what is left behind by living organisms, including bodily fluids, secretions and excretions. As a result, ATP will also be present in the broad range of biological soils found in the healthcare environment. ATP is an excellent cleaning marker because it is easily detected and measured, and it is a stable molecule, so it will not degrade after cells die and will not degrade in the environment before testing can be completed.[5]

Testing of cleanliness begins with obtaining a sample to be tested. Surface sampling is typically done with a device (most commonly, a swab) that will physically remove and capture residual soil from a surface. The device (e.g., swab) will be used on the surface after the surface is cleaned and dried. Some tests for sampling lumened medical instruments use a liquid forced through the lumen to remove and capture soil from inside the lumen. The collected liquid sample can then be tested. Detection and measurement of ATP in the sample is accomplished by using the same chemical reaction as that used by fireflies (lightning bugs) to create their "glow." The effect is called bioluminescence and is based on using the energy stored in the ATP molecule to create light. An enzyme called luciferase and an organic molecule called luciferin combine with ATP from the sample to create a chemical reaction where stored energy is converted

to light energy (See Figure 3). The amount of light created is a direct linear response to the amount of ATP present in the sample, so measurement of the amount of light produced becomes a measurement of the amount of ATP present and, hence, a measurement of the amount of residual soil.

Measuring equipment—called luminometers—is used to measure and quantify the amount of light created by the reaction. These devices utilize a sensor that detects the light (photons) and converts these into an electrical signal. A software algorithm then converts the electrical signal into a measurement. The unit of measurement used in ATP systems is the relative light unit (RLU). A key point to note in this phrase is the word "relative" as the RLU does not have an international or industry-wide definition. Each luminometer manufacturer may use a different sensor system and a different algorithm to create their version of an RHU (therefore, not all RLUs are created equally, and RLUs from different luminometer systems should not be compared). However, ATP system manufacturers should be able to provide the conversion factor between RLU and amount of ATP for their system (e.g., how many RLUs equate to 1 femtomole, or 1 X $10^{-15}$ moles, of ATP per square centimeter), so that the RLU result can be expressed in a standardized unit of femtomoles of ATP/$cm^2$.

Because the RLU is a direct result of the amount of ATP present and, hence, a result of the amount of residual soil present, the ATP RLU system provides a quantitative test of residual soil. Simply put, higher RLU values mean there is more residual soil than lower RLU values. Target RLUs that define an acceptably clean surface are often established and may vary depending on the ATP system used and the surface being tested (instruments, lumens, environmental surfaces, etc.). RLU numbers are also used to track quality process improvements over time.

## Objective 3: Compare ATP to other cleaning monitoring technologies

Several different biological compounds are used as cleaning markers. Some cleaning markers, such as carbohydrates or hemoglobin, are used when looking for a specific type of soil (e.g., hemoglobin as a test for residual blood) and are often used in conjunction with a test for other types of markers. Protein tests, however, are far more common and warrant a more detailed comparison.

Proteins are biological molecules that, like ATP, are present in all living cells; this makes protein a logical option as a cleaning marker. Residual protein can be quantitatively measured by laboratory tests, but these tests are time consuming and require specialized equipment. Protein-specific rapid tests are (as the name implies) fast and do not require sophisticated lab equipment, but they do not measure the actual amount of protein, only its presence above a baseline level. Rapid protein tests are based on a visual interpretation of a color change. Table 1 provides a more detailed comparison of rapid protein and ATP cleaning monitoring tests.

ATP systems provide a different type of test result than either rapid protein test systems or lab-based quantitative protein tests. ATP systems' RLUs are based on measurement of residual ATP. The lab-based protein test measures the amount of residual protein (typically reported in micrograms), but the rapid protein test only evaluates the presence of a defined amount of protein against a baseline based on interpretation of a color change. ATP and protein tests are not intended to be comparable. The cleaning markers used are different biological compounds, and the relationship between the amount of ATP and protein in any given residual soil will vary like the amount of ATP versus protein will vary in any given living tissue. Each technology is designed to provide cleanliness information based on the specific cleaning marker used by that system.

Another type of cleaning marker is sometimes used to evaluate the effectiveness of cleaning procedures on environmental surfaces. These are called fluorescent markers, but they are not naturally occurring components of soil. Instead, these markers are man-made chemicals that are artificially applied to an environmental surface before the surface is to be cleaned. These markers are only visible under ultraviolet (UV) light. The effectiveness of the cleaning process is evaluated based on a visual assessment (under UV light) of the amount of fluorescent marker remaining on the surface after cleaning. This type of system does not provide any data on the amount of biological soil on the surface, only a subjective visual assessment of how much artificial fluorescent marker may have been removed. ATP systems provide quantitative data on the cleanliness of the surface based on measurement of the remaining natural organic soil.

Microbial culturing of environmental surfaces or devices (lumens) is sometimes confused with cleaning monitoring. These tests use microbiological lab techniques to count the number of viable (living) bacteria in a sample, which are reported as colony forming units (CFU). The confusion related to cleaning monitoring derives from the fact that organic soils of human origin residing on an environmental surface may contain viable bacteria; therefore, it

| | ATP | PROTEIN |
|---|---|---|
| **Based on a universal cleaning marker** | Yes | Yes |
| **Provides a rapid test result** | Yes | Yes |
| **Uses a stable molecule as a cleaning marker** | Yes | Yes |
| **Provides quantitative (numerical) test data** | Yes | No |
| **Uses a standardized measurement scale** | No | N/A* |
| **Requires color interpretation of the test result** | No | Yes |

**Rapid protein tests do not produce quantitative (numerical) results.*

**Table 1 – Comparison of ATP and Rapid Protein Tests for Cleaning Monitoring**

is sometimes assumed that testing for bacteria may be an acceptable method for assessing the cleanliness of the surface. The problem with this approach is that cleaning is defined as the physical removal of soil, and bacteria are not a suitable marker for soil. The quantity and type of bacteria present in a soil will vary widely based on the specific origin of the soil. Culturing methods can only detect certain kinds of bacteria and can only detect viable bacteria. Many kinds of bacteria cannot survive in soil on a surface, so they will not show up in a culturing test. ATP is present in all soils of biological origin and is stable in the environment. The ATP measured in a soil sample came from all the cells—human and bacterial—present in the soil. For this reason, there cannot be any comparison of RLU measurements and CFU measurements. The RLU provides a broad picture of the amount of soil while the CFU presents a very narrow picture on the number of certain viable bacteria present.

Microbial culturing is also used to assess the effectiveness of reprocessing of flexible endoscopes. This testing is typically done after the high-level disinfection (HLD) process has been completed. As previously stated, HLD is not a cleaning process; it is a process designed to kill microorganisms remaining after cleaning. In this scenario, the principle of microbial culturing makes sense as the test result (number of viable bacteria) is aligned with the intent of the process it is testing (disinfection to kill bacteria). There should be caution in using ATP cleaning monitoring tests after the HLD process. Again, the ATP test is only providing information on the amount of organic soil in the sample, and it will not assess the number of viable bacteria—which is likely the more important information to have after the HLD process. The ATP measured at this point will come from all types of cells, so RLU results and CFU results will not align.

Thus, ATP testing is used as a measure of the adequacy of a cleaning process, whereas microbial culturing is used as a measure of the adequacy of a disinfection (i.e., killing) process. These are distinct and complementary tests that give different information about two different processes; therefore, one cannot be substituted for the other.

## Conclusion

Effective cleaning is an important element of any infection control plan. Rapid tests that evaluate the presence of residual organic soil by using chemical soil markers are a valuable quality control tool. ATP test systems are one option for quality control testing of cleaning processes. ATP is a universal soil marker that can provide rapid quantitative measurement of residual soil on environmental surfaces, medical equipment, and surgical devices. The RLU measurements can be used to assess cleanliness versus an established threshold or assess quality improvements made over time. C

### REFERENCES


1. Rutala W, Weber D. and the Healthcare Infection Control Advisory Committee (HICPAC) Centers for Disease Control and Prevention. *Guideline for Disinfection and Sterilization in Healthcare Facilities*. 2008.
2. International Organization for Standardization. *Sterilization of health care products — Vocabulary of terms used in sterilization and related equipment and process standards*. ISO 11139:2018. ISO, 2018.
3. ANSI/AAMI ST58: *Chemical sterilization and high-level disinfection in health care facilities*. Association for the Advancement of Medical Instrumentation. 2013.
4. ANSI/AAMI ST91: *Flexible and semi-rigid endoscope processing in health care facilities*. Association for the Advancement of Medical Instrumentation. 2015.
5. Alfa MJ, Fatima I, Murray B. Adenosine tri-phosphate (ATP)-based cleaning monitoring in health care: how rapidly does environmental ATP deteriorate? *J Hosp Infect*. 2015; 90: 59-65.