ATP Testing: Use and Misuse in the Restoration Industry

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Introduction

Measurements of cleanliness have leaped to the molecular level with the use of adenosine triphosphate (ATP) bioluminescence surface testing. When compared to visual examinations and microbial surface sampling, ATP analysis in the food, pharmaceutical, and medical industries offers a fast and dependable testing procedure to identify the presence of biological contaminants. Cleanliness validation of medical instrumentation and surgical equipment,[12] identification of trace body fluids and microbes in the food and beverage industry,[1] contamination among pharmaceutical products and equipment, and confirmation of water supply reservoirs are some of the industries that have benefitted from ATP testing. This paper examines the benefits and limitations of the ATP surface test method when applied to residential mold and bacterial damage restoration projects and examines ATP's practicality to "clear" homes during post-remediation verification (PRV).

Outside the medical, food, and pharmaceutical arenas, how much value do molecular test methods such as ATP bring? This paper will consider certain applications where ATP testing in residential homes damaged by a water loss may be appropriate and beneficial, what material surfaces are best suited for ATP testing, and inherent testing limitations that may cause misinterpretation of test results.

What is ATP?

Because this paper is about adenosine triphosphate, it is necessary for those who use the technique to have a basic understanding of what it is and how it functions in the living cell. ATP is an energy-transferring molecule that provides

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SYNOPSIS

ATP is a molecule that provides cellular energy for all living organisms. This paper examines the ATP surface test method when applied to residential mold and bacterial damage restoration projects and examines ATP's practicality to "clear" homes during post-remediation verification.

The ATP swab and swipe tests for the presence of ATP, an indication of microbial contamination because all living cells contain ATP. When the test swab is swiped over a contaminated surface, it accumulates organic matter containing ATP. The swab is then added to a lysing agent that breaks open the cells and releases the ATP. The release of light energy is measured using an illuminometer. The intensity of light is proportional to the amount of ATP.

Several circumstances can influence the reported concentration or interfere with ATP bioluminescence testing, including concentration sensitivity, temperature effects, disinfectants, enzyme-containing cleaning products, and surface soil.

Routine cleaning, followed by ATP surface measurements, provides an assessment of organic surface contamination and indicates cleaning efficacy, but those measurements should not be interpreted as a measure of microbial surface contamination. Cleaning is removal. Disinfection is a process of killing (not removing) biological contaminants. ATP testing does not distinguish dead from live biologicals and therefore may be an inadequate measure of disinfection.

Different microbes contain different ATP concentrations. Prokaryotic cells (bacteria) are single-celled organisms that range in size from 0.1–5.0 micrometer (µm) in diameter. In contrast, fungi, plants, animals and insects are eukaryotic cells that range in size from 10–100 µm. When ATP levels are compared between eukaryotic and prokaryotic cells, the larger eukaryotic cells contain higher ATP concentration.

When a sample is collected for ATP testing, the microbial population is not known. ATP results do not distinguish between fungi or bacteria, only that the light measurement was comparatively high or low. This finding has interpretive implications following a water loss where ATP measurements may initially reflect bacterial growth.

A hospital study compared ATP and the agar stamp method on various surfaces. The study initially revealed variability within each method. When the data was reevaluated, the prominent variable was the material surface properties. The ATP values from "high touch" surfaces were significantly different depending on the type of surface. There was no significant difference among the agar stamp values.

That finding was confirmed on polyvinyl chloride surfaces using a scanning electron microscope (SEM). The SEM revealed substantial surface roughness that allowed microbes to contaminate without being detected. The study concluded that the evaluation of cleanliness using ATP could result in an overestimate of cleanliness depending on surface conditions.

Wood materials are prone to fungal decay following sustained exposure to moisture. A study indicated that wood materials undergoing surface deterioration may express distorted ATP values as compared to wood materials that did not support fungal growth.

When a water loss occurs, initially bacteria are predominant. Researchers demonstrated that ATP concentrations depend on the bacterial species present and the growth stage. A restoration contractor cannot identify the growth phase of bacteria. Thus, ATP testing may offer an erroneous perspective on cleanliness.

Swab sampling poses prominent elements of collection variability. The action of swabbing is prone to variation. Restoration contractors should develop a swab sample collection protocol and sampling plan for ATP testing.

ATP surface testing is an effective method to evaluate cleanliness under the right circumstances. This paper examines the variables that influence ATP measurements and refines our understanding of this forensic tool so that ATP test results can be used purposefully and confidently.

cellular energy for all living organisms (*i.e.*, bacteria, fungi, insects, plants, and animals). All living organisms use ATP as the source of energy for basic cellular functions such as growth, reproduction, and cell maintenance. In the living cell, ATP production and energy release occur in the mitochondria (animal) or chloroplasts (plant) organelles. The energy produced by ATP drives all cellular activities and is necessary for all living organisms.

ATP is like a charged battery that discharges to form adenosine diphosphate (ADP) when cellular energy is needed. ADP can be recharged with the introduction of energy through the process of phosphorylation to form ATP thereby restoring the potential energy to do more cellular work, as shown in Diagram 1. Energy is released when a phosphate group (P) is released from ATP to form ADP.

How Does the ATP Bioluminescence Test Work?

The bioluminescence test works by measuring the light energy released from ATP when it releases a phosphate group. ATP contains three phosphate groups that are linked together by two high-energy bonds called phosphoanhydride bonds. When a phosphate group is removed to form ADP, energy is released (see Table 1). Energy is also released when a second phosphate group is removed from ADP to form adenosine monophosphate (AMP).

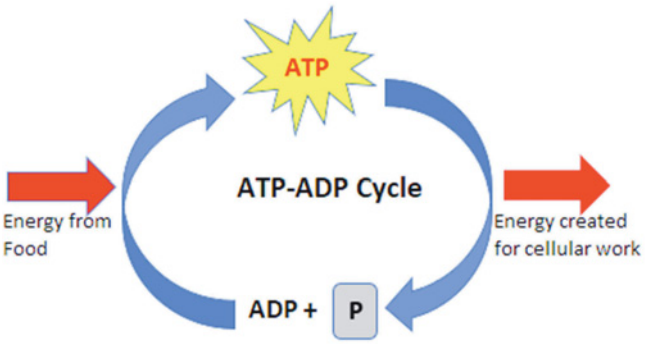

Diagram 1. ATP cycle showing conversion of ATP to ADP with the release of a phosphate group (P) and the release of energy; and then gaining a phosphate group back to form ATP. That chemical cycle provides the energy for all living cells.

Energy Release by the Removal of Phosphate Groups		
Reaction		ΔG [KJ/mol]
$ATP + H_2O$	$ADP + Pi$	-30.5
$ADP + H_2O$	$AMP + Pi$	-30.5
$ATP + H_2O$	$AMP + Pi$	-40.6

Table 1. Energy necessary to fuel cellular processes is obtained from the release of phosphate groups from ATP by hydrolysis (see addition of water in equation). That energy is used for reproductive and cellular processes.

The ATP swab and swipe tests for the presence of ATP, an indication of microbial (fungal or bacterial) contamination because all living cells contain ATP. The test is based on the energy released by the breaking of a phosphoanhydride bond (removing a phosphorous group from ATP). When you pass the test swab over a contaminated surface, it accumulates organic matter containing ATP. The swab with organic matter is then added to a lysing agent that breaks open the cells and releases the ATP. The lysed cells with exposed ATP are then mixed with a chemical called luciferase that catalyzes a reaction causing two phosphate groups to break off from the ATP molecules. The release of energy in the form of light is measured using an illuminometer that is part of the ATP test device and system.

An illuminometer measures the intensity of light which is proportional to the amount of ATP using a photodiode.[5] A photodiode is a semiconductor that converts the incoming light to an electrical current. The sensor conducts an electrical current directly proportional to the amount of light that it measures.

ATP Testing Limitations

Several circumstances can either influence the reported concentration or interfere with ATP bioluminescence testing.

- ***Concentration Sensitivity.*** Among four ATP bioluminescence devices studied (Hygiena, 3M, Charm, and Kikkoman) for accuracy and linearity, none of the meters expressed the same ATP reading for identical concentrations (see Figure 1).[16,17,18] Thus, from a cleanliness testing perspective, the same bioluminescence meter and swab sampling kit should be used for comparative measurements to limit sample variation.
- ***Temperature Effects.*** A 2001 study tested the thermal stability of ATP measurements using aliquots of cell lysate (disrupted cells) and suspended (unaffected

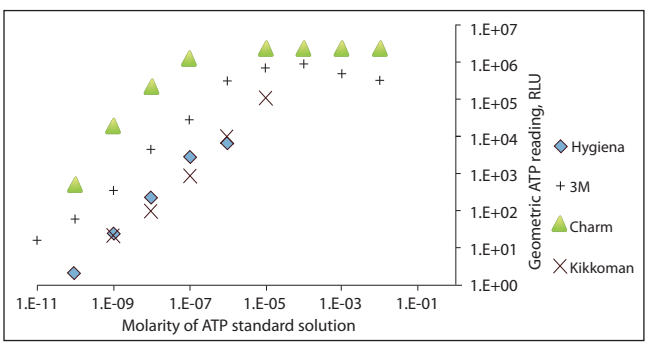

Figure 1. Testing showed ATP measurements from four different meters were linear at low concentrations but expressed lack of sensitivity and a flattened response at high concentrations.

cells) of E. coli.[3] ATP from the *disrupted* E. coli cells was purified, diluted, sealed in glass capillary tubes, and heated for varying periods in a water bath at a constant temperature of 60ºC (140ºF), and then cooled. The glass capillaries were crushed in a saline solution and an aliquot was analyzed for ATP. That analysis revealed that the cell lysate was thermally stable with similar results obtained when subjected to 90ºC (194ºF) for 10 minutes.

In contrast, suspensions of living E. coli cells that were subjected to 58°C (136°F) experienced complete ATP inactivation. The results demonstrated that the decreases in ATP content in living E. coli cells were much different than that expressed by the (E. coli) cell contents alone. The test results indicated that variation in ATP content in a living cell can be profoundly influenced by elevated temperatures. The authors concluded that when ATP measurements are taken with the bioluminescent method, the dynamic changes that occur in microbial ATP should be considered.

Those results suggest that when restoration contractors elevate the interior temperature of a structure to facilitate drying — a somewhat common practice — it may inactivate ATP and produce false-negative results and influence the conclusion of surface cleanliness.

- ***Interference from Disinfectants.*** Four ATP bioluminescence meters were evaluated to determine the sensitivity of each meter and the effect of cleaner-disinfectants on ATP measurements.[11] During the cleaner-disinfectant phase of the study, swab samples were exposed to the active ingredients in several commonly used disinfectants. Evaluation of the active ingredients was conducted to quantify and compare the effects of common disinfectant compounds. The products and active ingredients in each were:

  CaviCide: isopropyl alcohol.
  PCS 1000: sodium hypochlorite.
  Sanicloth: isopropyl alcohol.
  Accel TB: hydrogen peroxide.
  CleanCide: citric acid.
  Clorox H2O2 Wipes: sodium hypochlorite.
  70% IPA: isopropyl alcohol (lab sample).
  0.5% H2O2: hydrogen peroxide (lab sample).
  Clorox Ultra Bleach: sodium hypochlorite.
  Accel Prevention RTU: hydrogen peroxide.
  Virox 5 RTU: hydrogen peroxide.
  BTC50 (1:125): alkyl dimethyl benzyl ammonium chloride.
  Sporocidin: phenol.

The active ingredients lowered ATP measurements relative to a control concentration. The results from one meter (3M) are shown in Figure 2; however, all test meters showed similar variation from the control concentrations in response to the presence of disinfectants.

Whether used in hospital disinfection or a home restoration effort, the three most common ingredients — hydrogen peroxide (H2O2), sodium hypochlorite (chlorine bleach), isopropyl alcohol (IPA) — were present in 10 of the 13 common disinfectants.

Thus, in a water damage restoration setting, it would be a reasonable assumption that some tested surfaces that were absorbent (i.e., fabrics, cabinet surfaces, flooring, wood materials) would have been previously wiped with a disinfectant. Accordingly, disinfectant residue may remain (depending on the time frame and environmental conditions) as a residual chemical that could interfere with the ATP results.

- ***Enzyme Containing Cleaning Products.*** Cleaning products that contain enzymes as living or dead cells can greatly influence ATP readings. Care must be exercised to assure such products are not resident or remaining on the restoration test surfaces when ATP is being used for efficacy or clearance testing of the restoration work.
- ***Interference from Surface Soil.*** A soiled floor will interfere with the bioluminescence meter. The soil can block the light transmission so that an exceptionally soiled/contaminated surface can be read as non-problematic of biological contamination due to the blockage of transmitted light.

Interpreting ATP Measurements

Routine cleaning operations followed by ATP surface measurements provide an assessment of organic surface

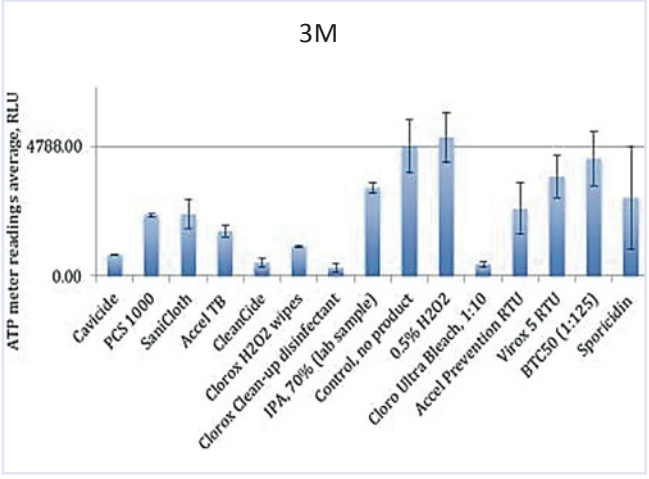

Figure 2. Interference of disinfectants on ATP measurements.

contamination and indicate cleaning efficiency. Those measurements should not be interpreted as a measure of microbial surface contamination.[14] Comparative studies using ATP surface samples and agar plate counts demonstrated that difference. When both ATP samples and microbiological plate counts were conducted together, the percentage agreement was approximately 70% in determining whether surfaces passed or failed[4]. Among the remaining 30%, sample variation between the two methods revealed that in most cases high ATP readings (implying ineffective cleaning and sanitizing) were accompanied by very low microbial plate counts. That difference was attributed to the presence of food residues that produced ATP measurements but were accompanied by low microorganism counts. Cases of low ATP and high microorganism counts were also revealed. Those discrepancies led to false-negative results.

Restoration and remediation of mold and bacteria are defined as the removal of biological contaminants. Cleaning is removal. In comparison, disinfection is a process of killing (not removing) biological contaminants. ATP testing does not distinguish dead from live biological contaminants and therefore may be an inappropriate measure of disinfection.

During restoration verification tasks, measurements used for cleanliness monitoring are intended to indicate viable microbial contamination. Unless the ATP measurements are accompanied by viable testing for living organisms to confirm the presence of active biological contaminants, the sampler cannot distinguish between insufficient cleaning efforts or nonviable (nonliving) organic residue.

ATP Differences Between Prokaryotes and Eukaryotes

All microbes do not share the same ATP concentrations. Prokaryotic cells (bacteria) are simple, single-celled (unicellular) organisms that lack a nucleus or any other membrane-bound organelle. The DNA in prokaryotic cells is found in the central part of the cell called the nucleoid. Prokaryotic cells are also distinguished by the composition of the cell wall. The bacterial cell wall is made of peptidoglycan that is composed of sugars and amino acids. Many bacteria are enclosed within a polysaccharide (polymer of sugar) capsule that provides protection by maintaining its shape and preventing dehydration. Bacteria attach to surfaces by means of the capsule making the surface more difficult to clean. Prokaryotic cells range in size from 0.1–5.0 micrometer (μm) in diameter.

In contrast, eukaryotic cells are more complex and have a distinct membrane-bound nucleus and other compartments called organelles with specialized cellular functions. Fungi, plants, animals, and insects are eukaryotic cells. The word eukaryotic means "true nucleus," and describes the occurrence of a membrane-bound nucleus in those cells. Eukaryotic cells range in size from 10–100 μm in diameter.

When ATP levels are compared between eukaryotic cells and prokaryotic cells, the larger eukaryotic cells contain higher ATP concentration. Among prokaryotes, gram-negative bacteria contain higher ATP concentrations than gram-positive bacteria.

When a swab sample is collected for ATP testing, the analyst has no idea of the microbial population being sampled. ATP sample results do not distinguish the presence of fungi or bacteria from one another, only that the light measurement was comparatively high or low. That finding has interpretive implications immediately following a water loss where ATP measurements may initially reflect prokaryotic (bacteria) growth.

Surface Properties and Measurement Irregularities

An ATP study on hospital cleanliness compared ATP bioluminescence and the agar stamp method on various surfaces.[15] The agar stamp or "food stamp" method detects the presence of bacteria on foodstuffs and working areas. It uses various types of agar media in tube form. The agar tube, sometimes referred to as a "sausage," is cut into slices and placed in a plastic holder that allows the sampler to securely press the exposed agar onto the test surface. After contact, a cap is placed over the agar and then cultured to reveal various bacterial strains.

The study revealed variability (n=752) using both methods. When the data was reevaluated, the prominent variable was the material surface properties. During the reevaluation, sample surfaces taken from the floor were omitted, allowing the remaining data (n=488) to be examined. The remaining surfaces were divided into six categories: melamine (n=63), vinyl chloride (n=16), stainless steel (n=144), wood (n=63), and acrylonitrile-butadiene-styrene resin-coated (n=48).

The ATP values obtained from "high touch" surfaces were significantly different depending on the type of surface. There was no significant difference among the agar stamp values. The data indicated that the accumulation of ATP depended more on the physical properties of the material surface such as electronic charges or surface contour and roughness.

That finding was confirmed on vinyl chloride material surfaces (unused hospital floor samples from each hospital tested) with observations made with a scanning electron microscope (SEM). The SEM examination revealed substantial surface roughness that allowed microbes to contaminate the material without being seen by the naked eye. The study concluded that the evaluation of hospital cleanliness using ATP value could result in an overestimate of cleanliness depending on surface conditions.

In a residential setting where wear and tear are common on many working surfaces, ATP sampling may not distinguish cleanliness on clean, smooth surfaces from contaminated, rough surfaces.

Surface Study Methods

In the study described in the previous section, the effect of surface features on the ATP results consisted of 752 surfaces within a nurse's work area. It included preparation tables, mobile workstations, corridor guardrails, hospital entrance floor, hospital room lockers, over-bed tables, and windowsills in both single and multiple patient rooms. Surfaces were wiped with a cotton swab; the agar stamp assay was derived from a soybean casein digest and cultured for 5–7 days under aerobic conditions at 30°C. The ATP values from high touch surfaces varied with the material property.

SEM of various comparative surfaces following ATP testing with each material surface revealed the trends shown in Figure 3. As you will note, stainless steel showed the lowest ATP values.

The findings indicated that the difference between the agar stamp values and ATP depended on the roughness of the surface. The study concluded that stainless steel was able to sustain cleanliness with a minimal attachment of organic constituents.

In a comparative evaluation, the study concluded that the ATP method determined that stainless steel was clean; however, the stamp agar method indicated the occurrence of microbial contamination. The reason behind that discrepancy was unknown; however, microbial attachment on stainless steel surfaces may be affected by temperature or humidity. Furthermore, if the microbes are allowed a duration of time to adhere and grow, stainless steel or polymeric materials (vinyl chloride) may offer a surface favorable for microbes to adhere and colonize. Overall, the data showed that ATP testing results may overestimate hospital cleanliness.

ATP testing on faced (melamine-coated), composite wood surfaces (particleboard) resulted in higher and more variable results than other hospital materials. Those results indicated that either faced, composite wood surfaces are more frequently "touched" or offered a more variable testing surface. The reason why ATP values on faced, composite wood surfaces were variable remains unknown. All composite wood material (particleboard) surfaces assessed in the

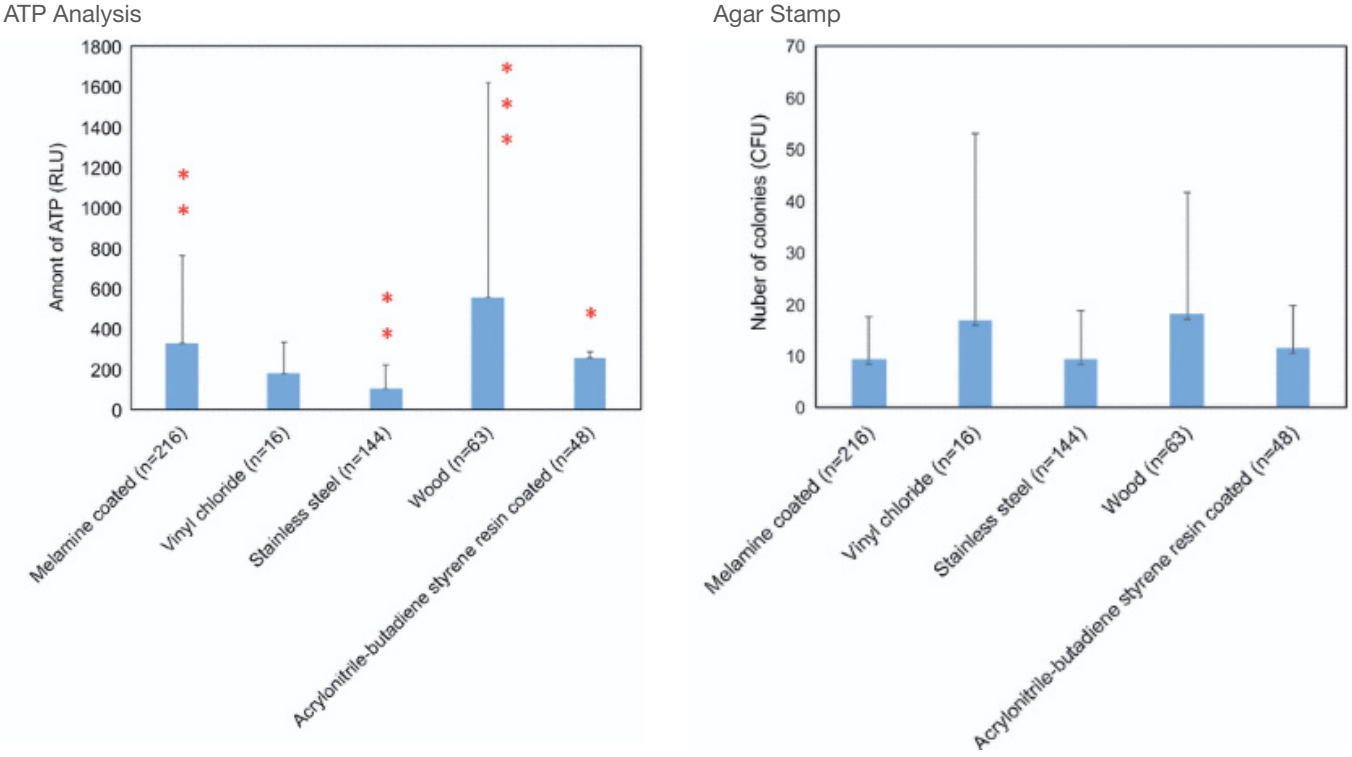

Figure 3. Comparison among microbial testing results using the ATP test and Agar Stamp methods on various cleaning surfaces.

study were coated with a melamine coating polymer as would be commonly found in a kitchen countertop or bath vanity. The difference of the polymer coating degree to protect wood surface could influence the ATP evaluation by changing the surface roughness and lessen microscopic areas of contaminant capture.

Contents coated with melamine are commonly used in tables, bed frames, dishes, and floors. Those materials prevent moisture absorption from spills, leaks, and contaminated materials and are assumed to be easier to clean and relatively free of microbial contamination.

The study results disputed that concept by reporting moderate ATP test values that indicated the influence of surface roughness and normal wear and tear modifications that allowed niches for microbial contaminants. Using SEM, surface images revealed prominent degrees of roughness on the melamine surfaces altered by wear. Those scratches and microscopic evidence of wear may not efficiently extract microbes using swab samples provided in the ATP kit and thereby offer limited data to assess cleanliness.

Surface Contamination and Wood Decay

Wood materials are prone to fungal decay following sustained exposure to moisture. Elevated ATP concentrations present in wood materials that undergo surface fungal deterioration may interfere with efforts to quantify cleanliness following a restoration effort.[10] In this three-month study, blocks of wood were inoculated with *Phanerochete chrysosporium,* a white rot fungus that specifically degrades lignin, leaving white cellulose behind. They were then sampled for nucleotide concentrations (ATP, ADP, AMP, and energy charge).

The study revealed that concentrations of ADP and AMP were 10 times larger than ATP as a result of deterioration. It indicated that wood materials undergoing surface deterioration may express distorted ATP values as compared to other wood materials that have been cleaned but did not support fungal growth. Thus, in restoration situations, ATP sampling of non-faced, deteriorated wood surfaces after cleaning may not be appropriate because a fungal-infested wood surface is rough, would likely capture contaminants, and distort the ATP concentration.

Variations in ATP Content with Bacterial Growth Phase

When a water loss occurs in a home, bacteria are the predominant organism that grows first and reproduces on exposed surfaces using available nutrients and moisture. Researchers were curious as to whether the concentration of ATP varied with the bacterial phases of growth (initial, log, stationary, death phases).[7] Levels of extracellular ATP in bacterial culture peaked around the end of the log phase and decreased during the stationary phase of growth.

The research demonstrated that ATP concentrations are dynamic depending on the bacterial species present and the growth stage. For example, *E. coli* and *Salmonella* were found to deplete extracellular (outside the cell wall) ATP. When an ATP supplement was added to the culture media, its effect was to enhance the survival of *E. coli* and *Salmonella* during the stationary growth phase. The research indicated that many bacterial species produced extracellular ATP during growth to enhance bacterial physiology.

A restoration contractor cannot identify the growth phase of bacteria. ATP surface samples may express a predominant growth phase immediately following a water loss or a range of phases on surfaces near and far from the water source. For that reason, ATP testing may offer an erroneous perspective on cleanliness depending on the sample location and duration of time following the loss.

Variations in the Swab Sampling Method

The swab sample is a common tool of the ATP test method. Swab sampling also poses prominent elements of collection variability depending on several conditions. The "pickup" or collection efficiency of the swab can vary based on the organisms present on the surface, the characteristics of the surface sampled, the moisture content of the surface, and the type of swab[6]. Critical to the ATP measurement is the amount of material recovered from the surface with the removal efficiency dependent on the physical characteristics of the material, the method and replicates used during sampling, and the initial wetness of the swab.

The action of swabbing is complex and prone to individual variation. There are at least eight variables that affect the collection efficiency of the swab: 1) the method of dragging the swab over the test and agar surfaces;[9] 2) the mechanical force applied to the swab; 3) the varying moisture content of the swab and the test surface;[9] 4) whether or not there is physical disruption of the microorganisms by the swab; 5) the degree to which the particulate mass adheres to the surface;[2] 6) the variable removal efficiency of different spores based on spore hydrophobicity (greater adhesion) and hydrophilicity (lesser adhesion) within the same genus;[13] 7) the roughness of the surface being sampled; and 8) the concentration of organisms present on the surface, with capture efficiency decreasing with increasing concentration.[8]

Restoration contractors should consider, at a minimum, the preparation of a swab sample collection protocol and sampling plan for ATP testing. That must be taught to all staff engaged in ATP testing to demonstrate formal training and uniformity in the sampling procedure.

Recommended and Not Recommended Applications of ATP Testing in Conjunction with a Disaster Restoration Project in a Residential Setting

The limitations posed by the ATP test method supported the following limitations in surfaces selected for sampling. The following are surfaces where ATP testing *is recommended:*

1. Hard, smooth surfaces: smooth or unworn plastic surfaces; stainless steel; aluminum; smooth, non-worn, vinyl or melamine-coated cabinetry; glass.
2. High touch areas in homes, especially of the immunocompromised.
3. Qualified food preparation areas.

The following are surfaces where ATP Testing is *not recommended:*

1. Gypsum board: Painted and unpainted.
2. Plaster.
3. Plastic: worn surfaces.
4. Solid wood materials: trusses, handrails, wood trim, rafters, floor sheathing and joists, plywood (interior, exterior, lauan).
5. Composite wood materials: unfaced particleboard, worn surfaces of faced particleboard, medium-density fiberboard, oriented-strand board, Masonite.
6. Flooring: wood (all), concrete, terrazzo, tile (all), carpeting (all).
7. Ceiling materials: plaster, popcorn ceilings.
8. Absorbent materials: fabrics, leather, bedding, furniture.
9. Worn/scratched metal surfaces.

Closing Remarks

ATP testing is an effective method to evaluate cleanliness in the proper setting. Outside of the medical and pharmaceutical applications, the ATP test method offers avenues for criticism and biased interpretation. That circumstance offers an excellent opportunity to A) investigate the variables that influence ATP measurements and B) refine our understanding of the forensic tool so that ATP test results can be used purposefully and, most importantly, justified and defended. JCS

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