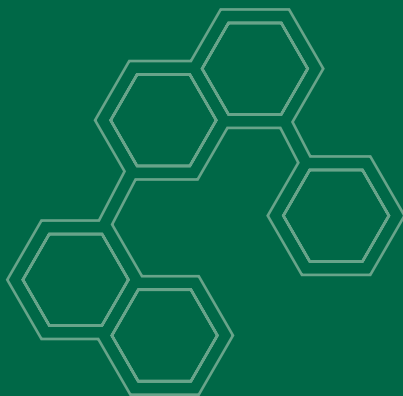




ATP HANDBOOK

Best Practices for Effectively Implementing
an ATP Sanitation Verification Program



Authors and Contributing Editors:

Dr. Robert Donofrio, Vice President Research and Development
NEOGEN

Andy Bohannon, Sanitation Product Manager
NEOGEN

Pauline Titchener, Senior Product Manager
NEOGEN

Alli Pearson, Project Manager
NEOGEN

Becky Michaels, Corporate Sanitarian
PepsiCo Global Food Safety

CONTENTS

Introduction	05
Section 1: Validation and Verification	06
Section 2: Cleaning to a Validated Standard	06
Section 3: Swabbing and Establishing Baselines	07
Section 4: RLUs as an Indication of Cleanliness	10
Section 5: Migrating From Validation to Verification	12
Section 6: Representative Sampling for Verification	12
Section 7: Pre-op vs Post-op	13
Section 8: Revalidation	13
Section 9: Using the ATP Test Results to Improve Cleaning Efficacy	15
Section 10: Interpreting Test Results: What's a Good Result	16
Section 11: Sampling Water	17
Section 12: Complementary Environmental Testing	18
Section 13: Program Extensions	20
Section 14: Limitation and Known Interferences	21
Section 15: Conclusions and Summary	22



INTRODUCTION

The establishment of a robust sanitation verification program is at the core of any food safety initiative. Adenosine triphosphate (ATP) sanitation verification systems have become the de facto standard used for measuring the effectiveness of cleaning efforts in the food production industry.

These systems measure ATP, a molecule produced in every living cell, to indicate the amount of cellular-based (a.k.a., organic) residue left on a surface after cleaning. While not necessarily a direct indicator of microbial presence, organic residues can contain allergenic proteins and/or product fragments that can contribute to off-taste in subsequent production runs. These organic deposits can serve as a reservoir for pathogenic microorganisms.

While ATP sanitation verification systems have commonly been used in production facilities, there have been little standardization on their applications. Many users have adopted simplistic practices in an attempt to simply have something and have missed out on the full value that can be derived from a strong monitoring program. There has also been a great deal of misinformation concerning the appropriate implementation and interpretation of results.

This handbook presents a best-practices approach to implementing an ATP system enabling the facility's sanitation verification program to provide more than just a basic pass/fail functionality. It seeks to assist with the establishment of a sanitation verification program that provides sanitation managers with accurate and appropriate information so that they can make the important decisions necessary to ensure the highest level of food quality and safety.

It should be noted that a robust sanitation verification program assumes that best practices for the prevention of contamination exist in the facility, such as the use of personal protection equipment, gloves, and hairnets.

SECTION 1: VALIDATION AND VERIFICATION

Validation is the process a facility undertakes to ensure the effectiveness of a particular operation. In this case, the process refers to a facility's materials and procedures used for cleaning after a production run.

The goal of the cleaning process in a food production environment is to effectively remove all particulates, residues, and microbial organisms to a safe and/or satisfactory level. Validation is proof that the goal can be achieved. It must be based on logical inferences and measurable results, and those results must be translatable to standards that can be used for routine monitoring during a normal production cycle.

Validation is typically performed until the expected outcomes are achieved and then repeated on a scheduled basis — or when the underlying assumptions used for validation have changed.

Verification is the routine monitoring of the process to determine adherence to the validated standard. This is typically done after each cleaning, and results are compared against the performance levels obtained during the validation process. Results that fall outside the validated standard indicate that one or more components of the cleaning process failed. A facility's verification process is typically incorporated into its Sanitation Standard Operating Procedure (SSOP).

SECTION 2: CLEANING TO A VALIDATED STANDARD

Cleaning in a food production environment is a critical base to any facility's food safety program. Cleaning failures can result in microbial, chemical (including allergens), or physical material contamination of future production.

The challenge for most food production facilities is in establishing objectives and standards that can be measured in a meaningful way. Since most food contaminants that can represent a safety issue for consumers are either microbial, chemical, or allergenic proteins, the optimal cleaning process will result when these entities are either removed or rendered inert. For that reason, most facilities use a cleaning process that focuses on the removal of the soil that can house these contaminants, followed by a sanitation step designed for microbial kill.

Step one in the validation process is determining the efficacy of the current cleaning program. In a wet-cleaned or clean-in-place (CIP) environment, this can be accomplished through ATP measurements directly following cleaning. If the facility utilizes a two-step clean and then sanitize process, measurements would typically occur following the cleaning but before the sanitation step to minimize the cost of sanitizing twice. The exception to this rule would be a sanitation step that enhances the removal of the soil.

The validation process requires the identification of test sites that reflect a representative sampling of the individual items being tested. As an example, product may flow along a production line through chutes and conveyors before arriving at a slicer and finally into a box or bag. Each unique surface that comes into contact with the product should be considered a unique test site. Harborage and difficult-to-clean areas should be a particular focus. It is also important to take into account non-food contact surfaces that could contribute to environmental contamination during production cycles.

Identifying a representative sampling site also implies the area that will need to be re-cleaned if it receives a failed ATP result. If, as an example, conveyor #1 is identified as a test site, any sample taken on any surface of the conveyor would be considered representative of the cleaning done for that entire piece of equipment. If a separate cleaning process is performed for any item of conveyor #1, it would imply a unique test site in the sample set. Once all test sites have been identified they should be recorded in a facility's SSOP and are typically referred to as the facility's test plan sites.

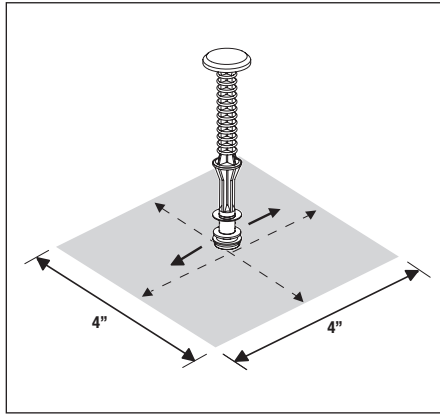
A SSOP must be adjusted to address sample sites that routinely fail to be cleaned effectively. Using the established RLU thresholds as a standard, a best practice approach to validating the SSOP would occur when the process achieves a passing result on three consecutive events.

SECTION 3: SWABBING AND ESTABLISHING BASELINES

Most ATP sanitation verification systems provide unique samplers or swabs for sampling surfaces, liquids, and in the case of NEOGEN's AccuPoint® system, the interiors of orifices. Each sampling device is designed for its intended use. Surface samplers or swabs contain an

extraction solution designed to optimally extract ATP from a surface. Since their sampling sponge or swab is saturated with the extraction solution, they should not be used for sampling water. Liquid samplers or swabs are dry and designed to absorb a specified volume of liquid.

When sampling a surface it is critical that a standard method be established and communicated to everyone who will be taking the samples. All commercial ATP systems are standardized on a 4" x 4" square for flat surfaces. The generally accepted procedure is to draw the square on the surface and then crisscross the sampler or swab in a tight weave pattern both horizontally and vertically within the square (figure 1). The importance of consistent sampling cannot be overstated for valid results.



Since ATP sampling can follow a wet-clean event, it is not unusual to encounter standing pools of liquid on surfaces. It is ideal to avoid these pools of liquid since they can serve to dilute the sample and hinder the absorption of the extracted sample.

For surfaces that cannot accommodate a 4" x 4" square, such as the interiors of nozzles, the best practices method is to establish a sampling pattern that approximates 16 square inches and can be easily replicated and communicated.

For areas that are inaccessible for routine sampling, such as CIP equipment, many facilities choose to sample final rinse water as an indication of cleaning efficacy. In this case it is important to standardize on the amount of liquid sampled and to use a sampler or swab specifically designed for liquid sampling. Surface samplers or swabs are typically saturated with an extraction solution on their tips and, therefore, do not absorb liquid appropriately. Liquid samplers or swabs are designed to absorb a defined, consistent volume of liquid thereby ensuring a valid sampling.



Inherent in any sampling program is the assumption that a surface has not been contaminated due to the sampling process. Since ATP is found in every living organism, including people, it is critical that those individuals performing the sampling not touch the surfaces they are about to sample. In addition to the obvious implications of contaminating a clean surface, altering the surface in any way, by wiping up a pool of water with a paper towel as an example, changes the surface and no longer appropriately represents the cleaning that was done.

SECTION 4: RLUS AS AN INDICATION OF CLEANLINESS

ATP systems deliver measurements in relative light unit (RLU) scores. The higher the number, the more organic soil that remained on the surface. The AccuPoint Advanced system's RLU range is 0–99,999. Many facilities establish a clean range, typically between 0–150, a marginal or cautionary range that reflects cleaning that falls within an acceptable level of variance at 151–300, and a fail range of 301 and higher.

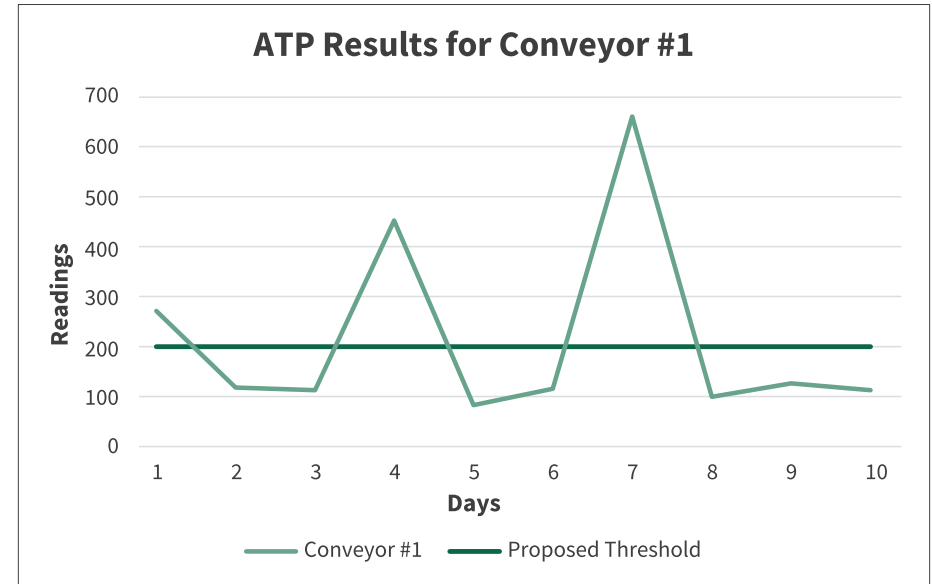
These ranges can vary significantly due to the real world requirements of the production process. Some establishments choose to not use a marginal or cautionary range and simply operate with a pass and fail protocol.

The pass threshold should represent the range of scores that would result from the successful completion of the cleaning process. In a wet clean and/or CIP scheme, the best way to make that determination is by monitoring your cleaning process to make sure it is followed correctly by performing a series of ATP measurements over several days or weeks to determine a representative sampling. At least 6–10 samples per test site will be necessary to determine the expected outcome. If a pattern is not established, continue sampling until enough evidence exists to make the determination.

An analysis of the data at that point should result in a performance curve where pass, marginal and fail thresholds will be evident. An example of ten test results for conveyor belt #1 is included below:

	Day									
Conveyor Belt #1	1	2	3	4	5	6	7	8	9	10
Reading	275	110	103	449	89	110	675	100	119	104

This data would suggest that when proper cleaning protocols are followed, typical results would range in the 0–120 RLU range as indicated from seven of the ten results. The results from days one, four, and seven indicate a failure in some aspect of the SSOP.

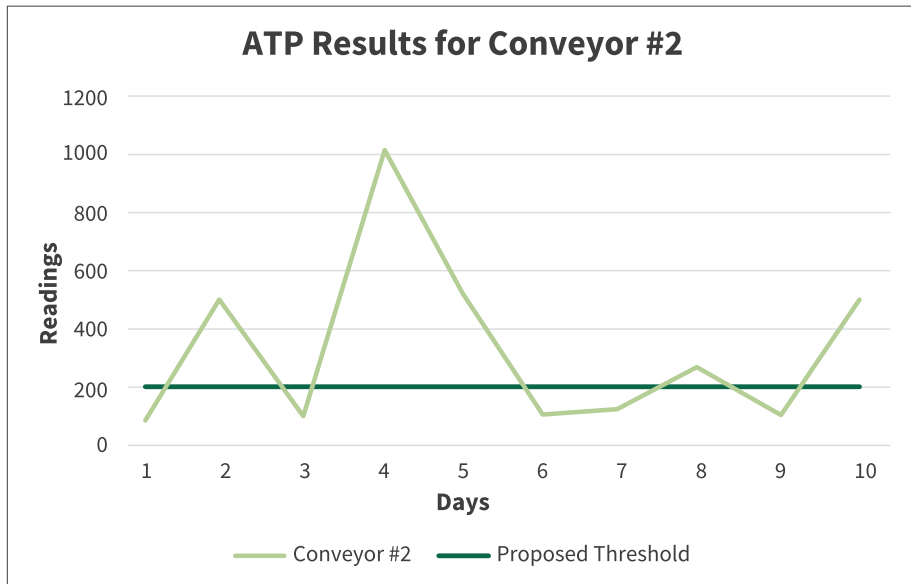


The data below suggests a different interpretation of the cleaning process for another piece of equipment, for example, conveyor chute #2:

	Day									
Conveyor Chute #2	1	2	3	4	5	6	7	8	9	10
Reading	75	446	109	1001	475	84	125	337	120	445

In this example, five of the ten samples resulted in a score of 125 or below. This gives some evidence to support a pass level of 125 RLU but also indicates a cleaning process that needs to be further investigated to understand the variation in results. In this case, more testing is needed after reviewing and monitoring the SSOP.

Once the pass, marginal, and fail baselines are established future process improvements can be evaluated relative to these standards.



SECTION 5: MIGRATING FROM VALIDATION TO VERIFICATION

Once the cleaning validation has been completed successfully, a facility will typically evolve their monitoring program to a more routine check of adherence to the validated standard. This verification is typically performed after each production run and associated cleaning event and is designed to determine whether the cleaning process was completed successfully relative to that standard. Since the census sampling and methods used during the validation process may be too cumbersome to perform on this routine basis, most facilities operate with a verification program that features representative sampling.

SECTION 6: REPRESENTATIVE SAMPLING FOR VERIFICATION

When developing a robust ATP-based sanitation verification program, the emphasis should be on establishing a routine sampling regimen that accurately reflects the cleaning that was performed following a production event. If a facility has identified 30 test sites for a production line, verification might be considered representative with a sampling of five or six sites.

The number of sites necessary to be considered representative can vary depending on a number of factors, but in general, the more consistent the test results, the fewer test sites required to be representative. If, as an example, three of the five test measurements result in a fail reading, larger sample sets may be appropriate. The number may also vary with a particular facility over time as cleaning processes are refined or fall out of specification.

SECTION 7: PRE-OP VS POST-OP

There are two primary uses for the verification data. In a post-operation (post-op) cleaning environment, the verification indicates that the previously produced material and any potential cross-contaminants have been removed from the production surfaces.

In a pre-operation (pre-op) environment, the test results are used as an indication that it is safe to begin the next production run. In many production facilities, the test results can be used for both, depending on the length of time between production runs and the environmental characteristics surrounding the production equipment. In general, the longer the time between production runs and the more activity around the equipment while it is idle, the more likely post-verification contamination has occurred.

As an example, if a production line sits idle after cleaning for eight hours and is near another production line producing a peanut and flour product, it's easy to imagine that the dust from that production would spread to the cleaned production line. In this example, the facility may shift their cleaning and verification programs from immediately after the production run to a time period closer to the next production run, could choose to segment the equipment to avoid cross contamination, or schedule a second, lighter version of their cleaning SSOP and verification pre-op.

SECTION 8: REVALIDATION

Validation should be repeated if an event has occurred that calls into question the assumptions behind the validated procedure. Events that would typically trigger the need to revalidate include:



- Changes in equipment layout or design including replacement equipment or additional equipment added.
- Environmental events such as flooding, fires, or other contamination events.
- Changes in cleaning chemical, supplier, process, or sanitation service provider.
- Changes in products produced.

SECTION 9: USING THE ATP TEST RESULTS TO IMPROVE CLEANING EFFICACY

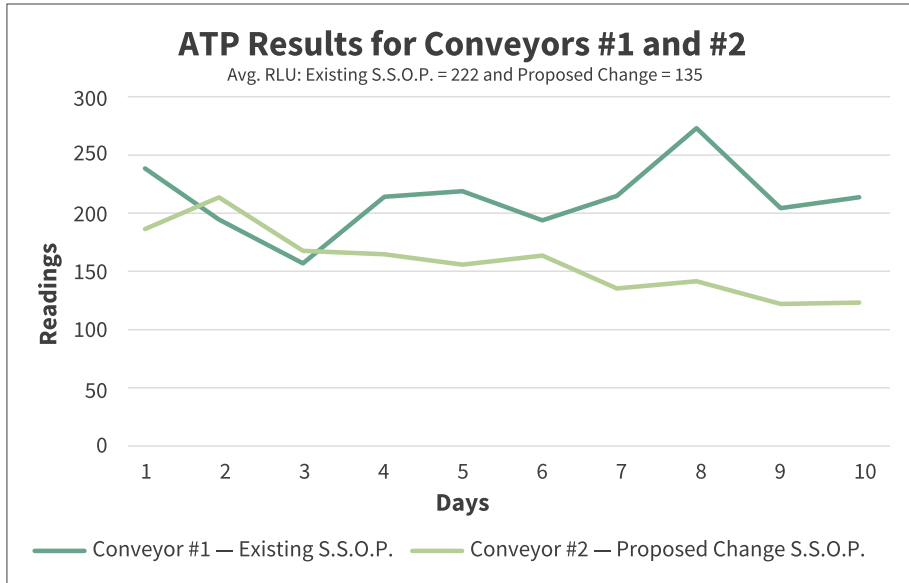
The optimal cleaning SSOP is one that provides the most consistent and effective removal of the organic soils. These two factors, effectiveness and consistency, are the critical factors in every sanitation program.

An ATP sanitation verification system can be an important tool for improving both the effectiveness and the consistency of the cleaning process. As noted above, the results from the sampling performed on conveyor chute #2 would indicate a SSOP that's inconsistent in its ability to remove the organic soil. An in-depth analysis of the cleaning process may indicate that the inconsistency comes from a variation in the products produced, e.g., a stickier product at some times and a product that's easier to clean than others. This may imply an adjustment to the SSOP for the stickier product.

Examination could also uncover cleaning behaviors that have resulted in the inconsistency, such as extending the effective use of the cleaning solution past the manufacturer's recommended levels. The test results provide an objective means with which to investigate where and why the inconsistencies may be occurring. Analyzing the ATP results alongside data from cleaning logs may allow for the identification of specific staff that are performing inadequate cleaning and thus the need for retraining, reassignment, or replacement. In effect, the ATP test results are a beacon shining a light on potential problem areas.

Once the SSOP has been refined and is providing consistent results, the ATP test results can be used to gauge the relative effect of process improvements. Changes to the cleaning chemicals, cleaning process, cleaning solution temperature, contact time, and any other variable

can now be gauged to determine more effective ways to remove the soil. This real-world data is invaluable when it becomes necessary to provide evidence to support additional expenditure requests to management.



SECTION 10: INTERPRETING TEST RESULTS: WHAT'S A GOOD RESULT

As discussed in the cleaning to a validated standard section, test results will vary between facilities for a number of valid reasons. Any suggestions of definitive thresholds need to be interpreted as typical or industry standards and not as absolutes for every facility. The process of interpreting test results must begin with establishing the facility's current situation. In effect, results need to be judged relative to themselves.

That can be accomplished through the method used for the establishment of effective testing thresholds as described above. This process is a validated appraisal of how well the facility's SSOP is doing now. The process then moves to stabilizing the results so that they are consistent with each execution.

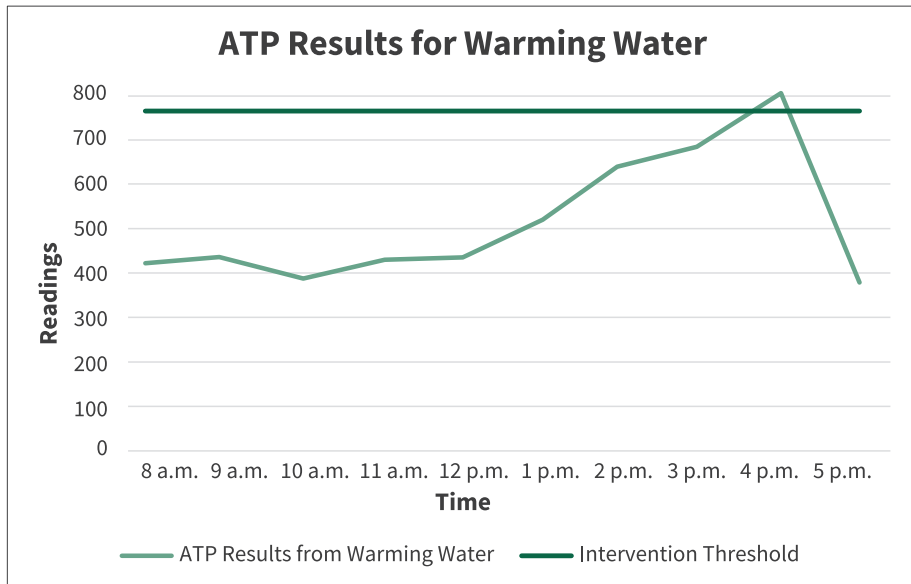
This second step usually results in the reduction of a greater number of scores, which indicates an improvement in cleaning consistency and an equilibration around a range of expected scores for successful cleaning.

The final step, which continues indefinitely, is to try new ways to improve the program and judge the results based on how they compare with the established thresholds. Improvements are identified, documented, and implemented when appropriate. New thresholds are set to reflect the new normal and the process is repeated on a routine, regular basis.

SECTION 11: SAMPLING WATER

The application of ATP systems for sampling water can be a valuable tool in gauging the organic load of the liquid. The key here is to establish a typical baseline, which may be much higher than for surfaces, and monitor the liquid on a scheduled basis to observe spikes. In this application, spikes would indicate the addition of organic material, such as the growth of microorganisms, and trigger an intervention such as the application of an antimicrobial solution (illustrated at right) or system maintenance such as steaming of activated carbon filters or replacing polishing filters. Spikes may also signal the failure of a process, a leak, or an overflow spill into the liquid. Some facilities establish a threshold for organic load that when reached, triggers the need to recirculate the water.

Understanding the baseline ATP level in water is critical to the evaluation of surface swab ATP levels. Cleaning procedures require a potable water rinse after application. Background ATP levels in the rinse water can contribute to the overall ATP on a given surface.



SECTION 12: COMPLEMENTARY ENVIRONMENTAL TESTING

ATP test results are critical tools for evaluating the ability of a facility’s SSOPs to remove soils from a cleaned surface. But they do not necessarily identify all potentially dangerous contaminants in the production process. In particular, allergen and microbial organism levels need to be measured and monitored as part of a robust environmental monitoring program.

Food allergens are proteins that elicit allergic reactions in some people. Because they are proteins and not ATP, they are not directly measured by an ATP monitoring system. Most often, they coexist with ATP sources in food but their presence may not directly correlate with ATP test results. They may also coexist at a level of concern, but still beneath the thresholds established with the ATP system. For these reasons it is important to supplement ATP sanitation verification with a robust allergen monitoring program if allergens are determined to be present in the facility. Handbooks are available from NEOGEN for developing an allergen control plan, and on best practices for food allergen validation and verification.



Microbiological monitoring is the third leg of a robust environmental monitoring program. Most often this is accomplished through a combination of general micro testing, such as for aerobic plate counts or total plate counts, and specific environmental pathogens such as *Listeria* and *Salmonella*.

General micro results will also often not correlate directly with ATP results due to a number of reasons, but the information obtained from this testing is critical for understanding the sanitary environment within the facility. A baseline value indicative of the current process must be established for these microbiological parameters as well. It is important to understand the primary role of the SSOP components on each of the contaminants in the facility. In general, microbial presence will be directly impacted through the facility's cleaning process, like ATP, but also greatly impacted by the sanitation and disinfectant step.

High readings with either ATP scores or micro results imply the need for review of a facility's SSOP. But, each measure provides a spotlight on a different component. Whereas high ATP scores suggest the need to review the cleaning portion of the SSOP and make modifications accordingly, the implication of a low ATP score and high micro count would suggest a need for review of the sanitation and disinfectant component of the SSOP.

SECTION 13: PROGRAM EXTENSIONS

An Audit Approach

An audit approach is appropriate when a large number of similar facilities require occasional, low frequency monitoring. Sampling is typically performed by a company or third-party auditor as part of a larger audit and reports are provided to management and staff. The critical features of this program are that:

- Audits are unscheduled so that test results can be considered typical and representative.
- Testing sites are only tested if clean. If an audit occurs during daily production, these sites may be significantly limited.

- Sites are selected at random with an emphasis on covering as many sites as are reasonable in the time allotted.
- Results are reviewed regularly and compared with like facilities.
- Sampling sites are standardized across all or most facilities.

Corporate Network Management

Corporations with multiple facilities have an opportunity to develop standardized programs for similar facilities with similar test sites and compare results to gauge performance. As with standardized production, equipment, SSOPs, and other programs, comparisons with corporate standards can be considered more valid.

In most cases, this approach requires advanced sanitation verification programs at each of the facilities. Data would be typically stored by each facility on the company's network and available for real-time review by corporate management.

SECTION 14: LIMITATIONS AND KNOWN INTERFERENCES

Most commercial ATP sanitation verification systems have incorporated buffers into their reaction solution to counter the pH effects of popular cleaning chemicals. However, extreme concentrations of cleaning solutions can overload the buffer and hinder the chemiluminescent reaction. This effect, sometimes known as quenching, can result in artificially low RLU readings. There have also been anecdotal reports of some ATP systems reporting artificially high results in the presence of peracetic/peroxyacetic acid solutions. These interferences should be discovered in the validation process but may appear if chemical concentrations change over time or with changes to the SSOP.

It should be noted that the chemistry that results in the chemiluminescent reaction, ATP + luciferin + luciferase, is an enzymatic reaction. As such, temperature can play a role in the amount of light that is produced. For most applications, this is not an issue. But in refrigerated or elevated temperature conditions, the effect must be considered in the interpretation of results. Generally speaking, temperature variations significantly beyond ambient temperature will typically lower RLU readings. The

effect will vary between ATP systems. Most manufacturers recommend equilibrating their samplers or swabs to ambient temperature before use.

While the temperature effect is an important consideration when interpreting ATP results, it must be considered in context. As long as the temperature between measurements does not vary significantly, the results will still be relative to each other. In other words, if the temperature in the sampling area is always 40°F, comparison of the results will be valid. A RLU score of 100 on Tuesday would be a valid comparison to a RLU score of 100 on Thursday. Where the temperature effect could render comparisons invalid is if the temperature on Tuesday was 40° and 75° on Thursday. If a facility wants to determine the temperature effect for its testing areas as part of the validation process the best practices recommended would be:

1. Take a series of samples, at least 6–10, at the test site locations and activate as instructed by the manufacturer’s specifications.
2. Take an equivalent number of samples at the test sites and:
 - a. label the samplers or swabs with the site location,
 - b. remove the samplers or swabs to a location at ambient temperature,
 - c. allow the devices to equilibrate for between 20 and 30 minutes,
3. Activate and insert into the ATP instrument as per manufacturer’s specification.

Compare the results of the two sample sets and adjust the thresholds appropriately. As an example, if the temperature effect appears to lower readings at the test site by an average of 20 RLU, the established threshold of 150 RLU may be more appropriately set to 130.

SECTION 15: CONCLUSIONS AND SUMMARY

ATP technology has been widely used over the past few decades in the food safety industry for the establishment and verification of cleanliness standards. This technology is attractive to the quality control and safety managers of food processing plants as it provides rapid and sensitive feedback regarding the state of sanitation for the surfaces being analyzed.

To maximize the usefulness of ATP in the food processing environment, implementing a standardized sanitation verification system is critical. This process includes identifying critical areas in the food production chain to include for analysis and establishing baseline measurements of ATP for the facility. The manager must take into account the sanitation practices of the facility and design the monitoring system to be in concert with the current practices. Once established, ongoing verification of plant sanitation via ATP monitoring will provide feedback relevant to the sanitary status of the plant. ATP data can reveal breakdowns in the administration of the sanitation practices as well as identify areas or surfaces of concern that may require additional remediation.

By understanding the limitations and interferences of ATP technology, as well as through the implementation of a properly designed ATP monitoring system, food safety sanitation managers can ensure the highest level of food quality and safety.

