LIGHTNING MVP**ICON**™

# Method Summary

BIOCONTROL
Results. Right now.

The **LIGHTNING MVP ICON™ is a pioneering system that measures and records ATP as well as other key HACCP parameters (pH, temperature, conductivity, and chemical concentration) and provides a management tool to ensure your program's success.**

ATP Monitoring

Adenosine triphosphate (ATP) is a molecule found in all living cells, therefore, it is present in most food residues. This makes it an ideal indicator of cleanliness in food manufacturing facilities. ATP found on surfaces in these facilities is primarily from an associated food residue but may also be from microorganisms such as bacteria or fungi. The quantity of ATP present on a surface serves as an indicator of the surface's cleanliness. As such, measuring ATP levels provides a direct indication of the effectiveness of the plant's cleaning practices. Ineffective cleaning can result in the build up of sufficient organic material to promote and support the growth of pathogenic bacteria or a variety of spoilage organisms. Routine monitoring of ATP levels at critical points allows food processors to optimize their cleaning and sanitation program to maximize its effectiveness at preventing these organisms from becoming established in the plant and proactively stopping quality problems before they start.

MVP ICON Detection Technology
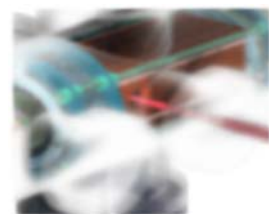
The MVP ICON sampling device is used to collect samples from a desired test point location. When the device is activated it releases the ATP present on the swab tip, combining it with the enzyme luciferin-luciferase. If ATP is present, the resulting reaction produces a bioluminescent signal (light) that is read and quantified

by the MVP ICON instrument. The amount of light emitted is directly related to the amount of ATP present in the sample. This allows the MVP ICON to instantly determine if a surface is sufficiently clean or if it is dirty and should be re-cleaned to prevent possible contamination of the product.

The MVP ICON utilizes state-of-the-art Photon Counting Sensor (PCS) technology, specifically developed to detect the light signals generated by small amounts of ATP at the photon-counting level. PCS technology provides the sensitivity and accuracy of a Photon Multiplier Tube (PMT) in a more compact lightweight design, allowing for the decrease in instrument size, weight, and power consumption. PCS technology provides the high performance needed to accurately assess the cleanliness of a surface.

Conductivity / Concentration

The MVP ICON Conductivity Probe provides two units of measurement within one probe. Cleaning and sanitizing chemical concentrations can be quickly measured and reported as parts-per-million (ppm). The built-in thermistor records temperature and provides automatic compensation to ensure concentration readings are accurate. The Conductivity Probe is ideal for auditing manual and automated chemical dispensing and delivery systems to ensure the effective cleaning and sanitizing of equipment and prevent the unnecessary waste and expense associated with preparing chemicals at higher than required concentrations.

The Conductivity Probe can also be used to provide a direct measure of cleanliness by measuring and recording the conductivity in microSiemens (µS) of process controls and clean-in-place (CIP) rinse water solutions. Simultaneous temperature readings provide the necessary compensation to ensure accuracy of the conductivity reading. The probe is easily calibrated on-site and all data is automatically recorded for tracking, and trending and analysis.

pH

The MVP ICON pH/Temperature Probe measures both the pH and temperature of solutions. The rugged, non-glass design allows for the probe to be used safely throughout the plant and laboratory. The probe provides built-in temperature compensation and Ion Sensitive Field Effect Transistor (ISFET) solid-state technology to ensure accurate pH readings.

Temperature

The MVP ICON Temperature Probe is a rugged, detachable probe that reads across a wide range of applications to make in-plant testing of product temperatures easy. The piercing tip design allows for easy penetration into food samples, allowing for an accurate measurement of the internal temperature rather than just surface temperatures.

MVP ICON Conductivity Probe Specifications	
Probe Technology	4-graphite electrode cell design, epoxy body
Conductivity Range	10 - 100,000 (µS)
Concentration Range	10 - 200,000 ppm
Temperature Range	0 °C - 100 °C
Resolution	Temperature 0.1 °C / Conductivity 0.1 µS /
Accuracy	Temperature ± 0.5 °C / Conductivity ± 1% /
Calibration	Single-point calibration
Temperature	2.5% / °C Non-selectable
Cell Constant	0.45 Non-selectable

MVP ICON pH/Temperature Probe Specifications	
Probe Technology	Ion Sensitive Field Effect Transistor (ISFET) solid-state pH sensor
pH Range	0.00 - 14.00 pH
Temperature Range	-5 °C - 105 °C
Resolution	pH 0.01 / Temperature 0.1 °C
Accuracy	pH ± 0.02 / Temperature ± 0.5 °C
Calibration	Three-point calibration at pH 4.00, 7.00 and 10.00
Temperature	2.5% / °C Non-selectable

MVP ICON Temperature Probe Specifications	
Probe Type	General purpose stainless steel temperature probe
Temperature Range	-20 °C - 105 °C
Resolution	0.1 °C
Accuracy	± 0.5 °C
Calibration	Single-point calibration

Neutralizing Sanitizers

MVP ICON sampling devices utilize proprietary chemistry specifically formulated to neutralize residual sanitizing chemicals, allowing surfaces to be accurately tested after sanitizing, if desired. In other systems, sanitizers can quench the luciferin-luciferase reaction resulting in inaccurately low ATP readings and the incorrect reporting of clean surfaces. Alternatively, sanitizers can cause artificially high readings, triggering unnecessary additional cleaning and sanitizing.

A series of side-by-side independent field trials were conducted by major food producers to compare the MVP ICON against three other ATP detection systems to determine the effect of sanitizers on the ATP detection reaction. Initial readings were generated using an ATP control containing a known amount of ATP. For comparison, a second set of readings were taken when the ATP control was combined with the producer's actual sanitizer.

In all trials, the MVP ICON's performance was superior to the competing system. The change in the MVP ICON's reading after the addition of sanitizer to the control ranged from –0.1 Zones to +0.5 Zones.

Competitive systems A, B, and C demonstrated a 73.8%, 100.0%, and 80.33% decrease in reading, respectively. Additionally, system B demonstrated as much as a 125.6% increase in reading.

Trial	Test Method	ATP Control	ATP Control + Sanitizer	Change in Reading	% Change
1	MVP ICON	2.2	2.1	- 0.1	3.0%
	Competitor A	15.25	4.00	-11.25	73.8%
2	MVP ICON	2.8	2.9	+ 0.1	3.0%
	Competitor A	46.5	37.7	- 8.8	19.0
3	MVP ICON	2.30	2.8	+ 0.5	23.0%
	Competitor B	1.3	3.0	+ 1.7	125.6%
4	MVP ICON	2.0	2.2	+ 0.2	7.5%
	Competitor B	2.0	0.0	- 2.0	100.0%
5	MVP ICON	2.9	3.2	+ 0.3	11.2%
	Competitor B	1.2	2.5	+ 1.3	113.7%
6	MVP ICON	2.3	2.4	+ 0.1	3.0%
	Competitor C	202.3	35.5	- 166.8	82.4%
7	MVP ICON	2.5	2.5	0.0	0.0%
	Competitor C	3121	614	- 2507	80.3%

Sensitivity

The precisely balanced enzymatic reaction of an activated MVP ICON sampling device combined with the advanced PCS technology of the MVP ICON instrument produces a highly-sensitive ATP detection system. There is a direct relationship between the amount of ATP present in a sample and the correlating Zone value generated by the ICON.

The MVP ICON system can detect and differentiate ATP levels as low as 0.5 picograms. However, field and laboratory trials establish surfaces for most environments are not truly dirty until the level of ATP reaches approximately 16-50 picograms. If desired, the Pass, Warn and Fail thresholds can be adjusted to suit a specific environment or hygiene monitoring goal, based on a user's own internal validation.

Correlation between Zones of Cleanliness and ATP Concentration
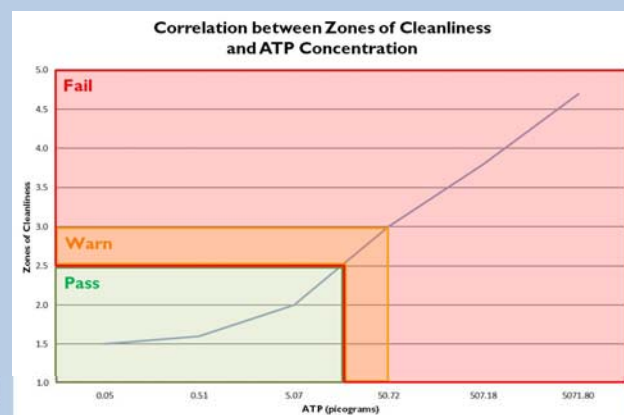
Zones of Cleanliness

Other ATP detection systems report the amount of light emitted by the luciferin-luciferase reaction as Relative Light Units (RLU). However, each ATP system's RLU scale is unique and cannot be compared to any other RLU scale. While the MVP ICON can display results as RLUs, the true value is found in the unique and easy-to-understand Zones of Cleanliness system employed by the MVP ICON.

All MVP ICON ATP results include a quantitative Zone value ranging from 0 to 5.0 Zones. Samples reading less than or equal to 2.5 Zones are considered clean and receive a "Pass" result. A "Warn" result is reported for samples between 2.6 – 3.0 Zones, which indicates the presence of trace amounts of ATP and the possible need for additional cleaning. Any reading $\geq$ 3.0 Zones will generate a "Fail" result, due to the presence of high amounts of organic material, and should be re-cleaned and re-tested.

The Zones of Cleanliness were established and validated through extensive laboratory and field trials, generating over 30,000 results from a variety of surfaces including stainless steel, rubber and plastic among a variety of food processing facilities.

Background - The baseline Zone value reading of the MVP ICON sampling device was determined in the laboratory by testing a minimum of 490 devices. The mean Zone value was 1.8 zones with a standard deviation of 0.16 Zones. Three standard deviations were applied to the mean to establish a 99.5% confidence level. This confidence level infers there is a 99.5% chance the background reading of an unused swab will fall between 1.3 and 2.4 Zones.

Sequential cleaning studies - Sequential cleaning studies were conducted in the laboratory to further validate the Zones of Cleanliness. Sterile stainless steel plates were contaminated with a variety of foods and microorganisms. The contaminated steel was cleaned using a variety of increasingly aggressive detergents and finally autoclaved. The steel was tested for ATP at each step of the process. The results showed effectively cleaned surfaces could produce Zone values equivalent to those produced by autoclaved surfaces, demonstrating the validity of the Zones of Cleanliness.

Field trials - A series of in-plant field studies were conducted in a variety of food processing facilities. Specific sites in each plant were tested after cleaning and sanitizing. The initial Zone values for these sites were in the Warn or Fail Zones. The same sites were monitored after post-production cleaning over a 2-5 day period. Plants such as pork slaughter, beef processing, and prepared salads all began at approximately 3.5 Zones but demonstrated a 25-fold improvement. All average Zone readings were lowered into the Pass Zone. In all cases, the Zone results from post-production cleaning decreased after only 2-5 days of ATP monitoring.

The Pass Zone was determined to be 0-2.5. The Warn Zone of 2.6-3.0 and the Fail Zone of 3.1-5.0 were established by placing three standard deviations between clean and dirty. The Warn Zone was established to account for any potential test variance and to ensure that, at the 99.5% confidence level, the clean and dirty levels did not overlap.
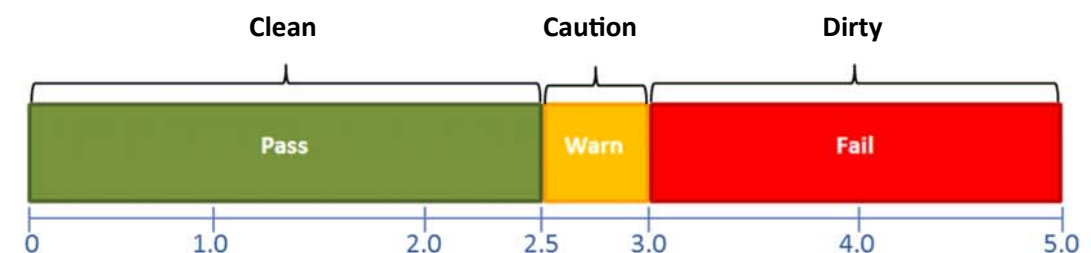
Sampling Device

The MVP ICON sampling devices have been specifically engineered to help ensure the accuracy of ATP readings. Ideally, sample collection results in ATP being deposited uniformly across the entire swab surface. However, variations in swabbing techniques and the non-uniform nature of surface contamination make this improbable. Additionally, most ATP detection systems attempt to read the light signal directly from only a portion of the swab surface, making it possible to obtain inaccurately low ATP readings, providing a false sense of cleanliness.

The MVP ICON sampling devices have been designed to minimize these effects. After collecting a sample, the swab is activated by depressing the plunger, forcing the reagents through the shaft and rinsing the sample from the swab tip to create a uniform sample solution. The bioluminescent signal is measured in the solution rather than attempting to read the swab surface. The result is a true determination of the amount of ATP collected from the surface and ultimately an accurate assessment of the cleanliness of the surface.
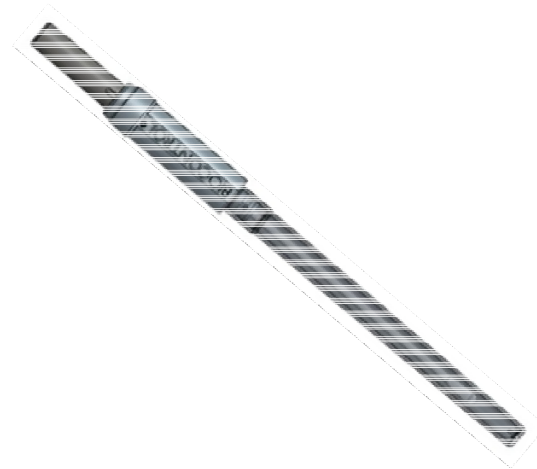
Any-time Verification and Calibration
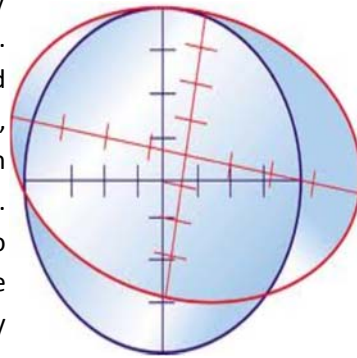
A requirement of most Hazard Analysis and Critical Control Points (HACCP) programs and Good Manufacturing Procedures (GMP) is the calibration of any process measuring equipment. The MVP ICON can be easily calibrated on-site by the operator for each of its measurement parameters (ATP, pH, temperature, conductivity, and concentration). The MVP ICON calibration rods for ATP include both a luminescent ^{14}C rod, that emits a constant low level of light, and a non-luminescent dark rod. The calibration set can also be used to verify that the MVP ICON is reading accurately. Calibration rod readings can be taken at any time and compared to expected values to ensure the accuracy of all readings.

LIGHTNING MVP ICON™ ATP Positive Controls, containing a known amount of lyophilized ATP, are available to verify the performance of the LIGHTNING MVP ICON™ Surface and Liquid Sampling Devices. The accessory probes for temperature, pH, conductivity and concentration are easily calibrated by following the simple touchscreen directions presented on the MVP ICON instrument. Calibration and verification results are recorded in the MVP ICON Dashboard software and are presented in the audit-ready HACCP reports.

MVP ICON Dashboard Software

Designed with quality management in mind, the MVP ICON Dashboard software provides a real-time snapshot of key program metrics essential to managing your HACCP program.

Re-test tracking - Know at-a-glance if failed test points are being properly re-cleaned and re-tested with the re-test dashboard widget and available re-test reports. Analyze re-test rates for specific sample plans, test points, and time periods, or simply identify the most high risk areas of the plant.

Swab usage - Set custom target testing rates for the month, quarter or year. Monitor activity against this goal to ensure the ATP testing program is being properly implemented and is in control.

Calibration status - Ensure the quality and accuracy of your hygiene monitoring program with built-in instrument calibration tracking. The MVP ICON instruments can be calibrated for all parameters on-site by the user. The dashboard calibration widget alerts users to each ICON's calibration due date.

Detailed Reports - The MVP ICON Dashboard software includes three categories of powerful, analytical reports. Action reports provide detailed analysis of the hygiene program's current performance. Trending reports compare current performance to past performance for a more detailed understanding of the program's effectiveness. HACCP reports provide QA Managers with audit-ready, print-and-present reports containing the key information required for internal and third-party audits.

Accuracy and Precision

The MVP ICON utilizes advanced, state-of-the-art Photon Counting Sensor technology and high-quality reagents to ensure the accuracy and precision of each ATP measurement. During validation of the system's performance five replicates of six different known ATP levels, including unused, activated sampling devices were tested. All replicates produced the correct Zone value for the corresponding ATP concentration, demonstrating the MVP ICON's high degree of accuracy. Additionally, the Standard Deviation (STDEV) for each group of replicates for all six ATP concentrations indicated virtually no variation between readings, demonstrating the precision of the MVP ICON's ATP measurements.

Accuracy and Precision of the MVP ICON's ATP Measurements

ATP Concentration	Precision		
	Replicates	Avg. Zone	STDEV
$1x10^{-11}$ Mol	5	4.8	0.055
$1x10^{-12}$ Mol	5	3.8	0.109
$1x10^{-13}$ Mol	5	3.0	0.114
$1x10^{-14}$ Mol	5	2.1	0.141
$1x10^{-15}$ Mol	5	1.6	0.303
0	5	1.6	0.268