A Guide to ATP Hygiene Monitoring
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1. Identify the location to be tested and turn on either the EnSURE or SystemSURE Plus luminometer. Remove either the Supersnap or Ultrasnap swab from the outer tube. Pressing firmly down on the swab tip, collect a sample from a 10 x 10 cm (4 x 4 in) area. Use a side-to-side and up-and-down motion while rotating the swab tip.

2. Place the swab back into the swab tube. The Supersnap or Ultrasnap device is now ready to be activated or can be left inactive for up to 4 hours in this state.

3. To activate, break the plastic valve at the top of the device by bending backward and forward. Squeeze the bulb twice to expel the liquid in the bulb to the bottom of the tube.

4. Bathe the swab bud in the liquid by shaking gently in a side-to-side motion for 5 seconds.

5. Place the entire swab device into either the EnSURE or SystemSURE Plus luminometers and close the lid. This step must be done within one minute of activation.
6. Holding either the EnSURE or SystemSURE Plus luminometers in a vertical position, press 'OK' to initiate reading. The test result will appear on the screen in 15 seconds.

Flow chart: Cleaning, Testing and Corrective Action Procedures
INTRODUCTION

The EnSURE & SystemSURE Plus ATP Detection Programs

In today’s market, impeccable hygiene control is an increasingly critical issue for industries involved in the manufacturing and distribution of consumable products.

Scigiene’s EnSURE & SystemSURE Plus ATP Detection Programs offers a state-of-the-art solution for organizations looking to advance the hygienic status of their facilities.

Recognized worldwide for accuracy, ease-of-use and affordability, the EnSURE & SystemSURE Plus ATP Detection Systems are used extensively throughout the food and beverage processing industries, as well as healthcare and pharmaceutical industries, hospitals, restaurants, supermarkets and other facilities where rapid detection of contamination is crucial.

The EnSURE & SystemSURE Plus ATP Detection Systems enables organizations to:

- Instantly assess the cleanliness of production surfaces, allowing immediate corrective action to be taken
- Reduce the use of conventional microbiological testing methods that are slow, labor intensive and costly
- Monitor water quality
- Enhance sanitation training, as well as verify efforts of sanitation personnel
- Record and track test results to identify problem areas, make improvements and show due diligence and compliance with HACCP, Sanitation Standard Operating Procedures (SSOPs) and industry regulations
- Optimize the use of sanitation chemicals
- Protect brand image by increasing product quality and avoiding recalls
The guidelines detailed within this manual are applicable to multiple business sectors, including but not limited to:

- Food and Beverage industry
- Food Service
- Hospitality
- Laboratories
- Government
- Education
- Pharmaceutical and Healthcare fields

Components of the EnSURE & SystemSURE Plus ATP Detection Systems

The EnSURE & SystemSURE Plus ATP Detection Systems includes three components:

- **The EnSURE or SystemSURE Plus Luminometer** – a user-friendly, handheld light-reading unit that provides precise, on-site hygiene test results. Used with either the Supersnap or Ultrasnap sample testing devices, extremely low levels of contamination can be detected in just 15 seconds.

- **Supersnap or Ultrasnap Sample Testing Devices** – are convenient, all-in-one ATP sampling device that is activated with a simple swab, snap and squeeze action.

- **SureTrend - Data Analysis Software** – a powerful software program that allows users to upload test results to a database, analyze trends and generate reports.

Scigenie’s luminometers, sample testing devices and software are all designed for ease-of-use and reliability, enabling both technical and non-technical staff to operate both the EnSURE & SystemSURE Plus ATP Detection Systems without difficulty.

Overview of this guide

This guide is designed to assist users in setting up and operating their own ATP hygiene monitoring program using either the EnSURE or SystemSURE Plus ATP Detection Systems. The contents of this manual include:

**Quick Start Guide**

A quick reference on how to use the EnSURE & SystemSURE Plus ATP Detection Systems, the Quick Start Guide is included at the beginning of this
Section I: An overview of the ATP Detection Program
This section discusses what ATP is, how it’s used in rapid hygiene bioluminescence testing, as well as the benefits of an ATP monitoring program and other applicable uses for the EnSURE & SystemSURE Plus Systems.

Section II: Using your EnSURE & SystemSURE Plus ATP Detection Systems
This section provides step-by-step practical instructions for how to use the EnSURE & SystemSURE Plus ATP Detection Systems as part of your hygiene monitoring program. It includes information on how to properly collect and test samples, where and when to do ATP testing, determining Pass/Fail levels, taking corrective action, and using the Sure Trend software package to manage and track test results over time.

Section III: Further help
Section III offers Troubleshooting information, a Glossary of Terms, useful Appendices, as well as information on how to contact Scigiene – Hygiena’s Canadian Master Distributor.
**SECTION 1: An overview of the EnSURE & SystemSURE Plus ATP Detection Programs**

The EnSURE & SystemSURE Plus ATP Detection Programs are rapid hygiene monitoring programs designed to help organizations achieve optimal hygiene status within their facilities. The Program combines the three-part ATP Detection System with a comprehensive monitoring plan to test, record and track biological contamination on production surfaces and in water samples.

A key feature of both the EnSURE & SystemSURE Plus ATP Detection Systems is the use of bioluminescence technology to identify and measure adenosine triphosphate, commonly known as ATP.

**1.1 What is ATP?**

ATP is an energy molecule found in all plant, animal and microbial cells. It fuels metabolic processes such as cellular reproduction, muscle contraction, plant photosynthesis, and respiration in fungi and fermentation in yeast. All organic matter (living or once-living) contains ATP, including food, bacteria, mold and other microorganisms. The detection of ATP on a surface therefore indicates the presence of biological matter that may not otherwise be visible to the eye.

In industries where hygiene control is crucial, ATP testing is an excellent tool for detecting and measuring biological matter on surfaces and in water. Scigiene’s EnSURE & SystemSURE Plus ATP Detection Systems use ATP bioluminescence technology to measure extremely low levels of organic residue.

**1.2 Measuring ATP with bioluminescence technology**

The Supersnap & Ultrasnap ATP Sample Testing Device contains a natural enzyme found in fireflies. This enzyme, called luciferase, produces a simple bioluminescence (light-producing) reaction when it comes into contact with ATP. Using bioluminescence technology, the EnSURE & SystemSURE Plus luminometer can measure extremely low levels of ATP collected with the Supersnap & Ultrasnap devices.

Measuring the amount of bioluminescence from an ATP reaction provides a good indication of surface cleanliness or water quality.
because the quantity of light generated by the reaction is directly proportional to the amount of ATP present in the sample. The bioluminescence reaction is immediate so results can be processed in seconds, at the testing site, by inserting the swab device into either the EnSURE or SystemSURE Plus unit. Results are then expressed numerically on the luminometer’s screen as Relative Light Units (RLU).

1.3 Interpreting Results on the EnSURE & SystemSURE Plus Luminometers

The relationship between the amount of ATP on the sample and the RLU result reading on the luminometer is simple:

- **High contamination (improper cleaning)**
  - Large amount of ATP
  - More light produced
  - High RLU reading

- **Low contamination (proper cleaning)**
  - Small amount of ATP
  - Less light produced
  - Low RLU reading

The RLU reading is directly proportional to the amount of ATP collected from the sample. A high RLU reading indicates a large amount of ATP at the test location. This in turn indicates improper cleaning and the presence of contaminants.

Cleaning properly results in less ATP at the location. Less ATP results in less light output during the bioluminescent reaction and consequently, a lower RLU reading.

1.4 Benefits of routine ATP testing

Routine ATP testing with the EnSURE & SystemSURE Plus ATP Detection Systems offers a number of benefits to the end user:
Saves money and time by:

✧ Ensuring efficient cleaning processes, which reduce the need for labor-intensive re-cleaning while minimizing the amount of expensive sanitation chemicals needed to properly sanitize equipment

✧ Providing rapid results on-site, allowing for immediate corrective action that can prevent expensive product recalls and delayed production

✧ Reducing the required amount of environmental monitoring, lab costs and labor expenses

Provides brand protection by:

✧ Increasing product quality and shelf-life

✧ Preventing food-borne illness and health violations that can compromise the brand image

✧ Demonstrating to clients and auditors that food safety is a priority

**1.5 Other applicable uses for the EnSURE& SystemSURE Plus ATP Luminometer Detection Systems**

In addition to routine ATP hygiene monitoring, there are also other useful applications for the EnSURE & SystemSURE Plus ATP Detection Systems:

✧ **Troubleshooting** – ATP testing with your EnSure or SystemSURE Plus provides a way to expose microbial contamination and other issues that might be causing higher than normal plate counts in environmental testing. It may also be useful in determining the factors affecting end-product screening results (such as product inconsistency or spoilage).

✧ **Randomized testing** – The EnSURE & SystemSURE Plus systems can be used to audit cleaning efficacy by doing random spot checks of designated testing locations. A large number of test locations are set up. Of these locations, a select number are tested daily or weekly on a randomized or rotating schedule.

✧ **Production line disassembly** – During a routine full line disassembly and reassembly, it is recommended to do copious amounts of ATP testing to find and clean all areas of machinery that have the potential to harbor harmful bacteria.

✧ **Validation of hand cleanliness** – ATP testing can be used to verify proper washing techniques and cleanliness of employees’ hands.
when used directly on skin. When doing this type of testing, it is important to identify appropriate pass/fail levels taking into account naturally occurring ATP levels in skin cells. To find out more about using ATP testing to verify hand cleanliness, contact Scigenie Corporation.

Training – The EnSURE & SystemSURE Plus ATP Detection Systems are an effective tool for teaching sanitation crews proper cleaning procedures, since it gives them on-the-spot feedback.

SECTION 2: Using your EnSURE & SystemSURE Plus ATP Detection Systems

Proper sampling at critical test locations, correct use of the luminometer and sampling devices, and accurate data management are crucial components of the EnSure & SystemSURE Plus ATP Detection Programs. Consistency in the procedures used ensures accurate results. Effective record keeping and documentation of results allows for helpful analysis and data retrieval during an audit.

This section will explain how to:

✧ collect a sample using the Supersnap & Ultrasnap devices
✧ use the EnSURE & SystemSURE Plus luminometers to measure ATP
✧ determine where and when to test control points
✧ set Pass/Caution/Fail thresholds
✧ follow corrective action procedures
✧ upload results to the Data Analysis database for storage and analysis

2.1 Proper sampling procedures

Before collecting a sample for testing, the surface should be visibly clean. If any soiling or residue is apparent, re-clean the work area before testing.

2.1.1 Collecting samples with the SuperSnap & Ultrasnap device

1. Remove the Supersnap or Ultrasnap device from the package. Next, remove the outer tube by holding onto the double ring base of the Snap-Valve while pulling down on the tube. The swab tip comes pre-moistened with a detergent that breaks down biofilm* on the test surface. Condensation may be visible on the inside of the swab tube. This is normal. **Do not touch the swab tip or shaft with fingers or anything**
else, as this will contaminate the test. Discard any swabs that accidentally get tainted or activated.

NOTE: For optimal performance, swabs that have been removed from cold storage should stand for 10 minutes at room temperature before use.

2. Collect a sample with the Ultrasnap device using the guidelines below. Be careful not to overload the swab bud with too much sample. The Supersnap and Ultrasnap devices are designed to detect trace amounts of contamination, therefore no sample residue should be visible on the swab. An excessive amount of sample may interfere with the bioluminescence reaction and produce an inaccurate test result.

* For a definition of biofilm, please see the Glossary section of this guide.

**a) Regular surfaces**

Swab a 10 x 10 cm (4 x 4 in) square on the test surface. Rotate the swab as the sample is collected, while applying firm pressure to break through any biofilm. This will create a slight bend in the swab shaft.

**b) Irregular surfaces**

Where 10 x 10 cm square sampling is not feasible, swab as much of the surface as possible. Be sure that a slight bend in the shaft is achieved and an adequate sample is collected.

**c) Water sampling**

Dip the swab for 5 to 10 seconds into a 20 mL sample of the water being tested. If the water is not homogenous or contains sediment, mix it thoroughly before sampling.

NOTE: Supersnap & Ultrasnap swabs are designed primarily for surfaces, but can be used for some types of water samples. Contact Scigiene to find about the Aquasnap for water testing.
3. Re-insert the swab into the tube. The Supersnap or Ultrasnap is now ready to be activated, or it can be left inactive for up to 4 hours in this state. In some facilities, users prefer to sample each location, write the sample location on the swab tube and run all tests in a laboratory rather than at the test location, or they may choose to run each test right after collecting their sample.

If testing occurs at the sampling location, turn on the EnSURE or SystemSURE Plus luminometer and scroll through the program numbers (PROG) to find the one that correlates to the location being tested. This action can also be taken prior to swabbing. This is up to the user. For more information, see Establishing your facility's test locations, section 2.2.1.

4. Holding the device upright, activate the Supersnap or Ultrasnap by bending the bulb at the top until the plastic Snap-Valve breaks, then bend once more in the opposite direction. Squeeze the bulb twice to expel the liquid-stable reagent contained in the bulb and allow it to flow to the bottom of the tube.

5. Gently shake the device with a side-to-side motion, bathing the swab bud in the liquid-stable reagent. The test is now activated and the bioluminescence reaction is taking place. For optimal results, the reading should be taken on the EnSure or SystemSURE Plus luminometer within 5 to 60 seconds of activation.

2.1.2 Measuring ATP with the EnSURE & SystemSURE Plus luminometers

1. Open the lid on the EnSURE or SystemSURE Plus Luminometer, and insert the activated Supersnap or Ultrasnap device into the reading chamber. Close the lid, making sure to keep the machine upright.
2. Press “OK” on the Ensure or SystemSURE Plus to initiate measurement of RLU output. Results are displayed on the screen in 15 seconds.

### 2.1.3 General precautions and warnings

- If the Supersnap or Ultrasnap device accidentally gets activated, do not use.
- Avoid collecting large amounts of sample on the swab bud.
- Hold the EnSure & SystemSURE Plus upright when taking readings.
- Hold the Supersnap or Ultrasnap device upright when activating.
- Read the Supersnap or Ultrasnap sampling device within one minute of activation.
- Keep the Supersnap or Ultrasnap device out of direct light when possible.
- To ensure shelf-life for up to a year, the Supersnap and Ultrasnap sampling devices should be refrigerated at 2-8 °C (35-46 °F). Sample devices will tolerate room temperature (<25 °C) for 4 weeks.
- Refrigerated sampling devices should be left out at room temperature for 10 minutes before being used.
2.1.4 Ensuring correct results with calibration controls

To help ensure the accuracy of test results, the EnSURE & SystemSURE Plus luminometers are programmed to automatically self-calibrate every time the instrument is turned on. During the 60-second calibration, it checks the light sensor and adjusts to temperature and humidity.

To further verify test result accuracy, Scigiene offers two calibration kits that are recommended for periodic use with your EnSURE & SystemSURE Plus ATP Detection Systems: the Calibration Control Rod Kit (Part # ATP4000) for testing the luminometers, and the Positive Control Kit (Part # ATP2500) for testing Supersnap & Ultrasnap devices.

Calibration Control Rod Kit (Part # ATP4000)
It’s recommended that you verify calibration of the Ensure & SystemSURE Plus luminometers with the Calibration Control Kit once a month. Incorporating the Calibration Control Kit into your overall Quality Control program will confirm that the instrument is within specifications and operating correctly.

Each kit contains a Positive rod and a Negative rod. The Positive rod emits a very low level of radiation, producing a constant light output that can be measured in RLUs to verify proper calibration of the unit. The Negative Rod produces low-light or zero (0) RLU and is used to check that background light is not entering the instrument, while ensuring that the light detector is calibrating correctly.

For instructions on how to use the Calibration Control Rod Kit, as well as how to interpret results, consult the product insert.

Positive Control Kit (Part #ATP2500)
The Positive Control Kit is used for validating the efficacy and quality of the Supersnap & Ultrasnap ATP Sample Testing Devices. It comes with 25 sealed glass vials, each of which contain an identical amount (approx. $5 \times 10^{-13}$ moles) of freeze-dried ATP and sugars to provide a predictable result if the Supersnap and Ultrasnap devices are used and stored correctly.

Each vial provides a sample which produces a positive result when tested with the Ultrasnap. Positive Controls should be used to test one device in each bag of 25 devices or whenever there is concern about the product’s storage temperature or shelf life. Incorporating the Positive Control Kit into the overall Quality Control program will validate results of the EnSURE & SystemSURE Plus and the Supersnap & Ultrasnap.
For instructions on how to use the Positive Control Kit, as well as how to interpret results, consult the product insert.

2.2 Where to do ATP testing

2.2.1 Establishing your facility’s test locations (control points)

Before sample tests are taken, you need to identify areas within your facility that are at risk for contamination from product residue or microbe growth. These areas should be designated as “control points” in your hygiene monitoring plan. A control point should provide reliable feedback on the cleanliness of a particular piece of equipment or area during testing. It’s important that ATP testing is routinely performed on all control points, including product contact areas (direct and indirect) and hard-to-clean sites on your processing and handling lines.

- If you currently have HACCP or SSOP programs in place, or if you conduct environmental testing, you may choose to use the same test locations established for these programs as your control points for ATP monitoring.

- If you haven’t previously established test locations, you need to determine where your facility’s areas of contamination risk are. This can be done by swabbing multiple areas on equipment and production line surfaces after routine cleaning. ATP levels will be higher in those spots that are harder to clean, spots that are missed in your current cleaning procedure, and spots that have developed biofilms. These areas should be established as control points for routine testing and monitoring.

2.2.2 Types of contact areas

Your ATP testing plan should include the following locations:

a) Direct contact areas

Direct contact areas are surfaces where the presence of any contaminant will taint the product. These are high-risk areas that should be tested frequently.

Examples: conveyor belt, wash bucket, cutting board, slicers and utensils

A direct contact surface (such as a conveyor belt) should be tested in a few different places to verify total cleanliness.
b) **Indirect contact areas**

Indirect contact areas are locations where splashed product or contaminants can be dropped, drained, or transferred onto the product. These areas are often overlooked as sources of contamination and should be routinely tested.

*Examples:* sink, drain, side walls and additional machine parts

Indicated areas of the sink are examples of indirect contact points where ATP testing should be conducted.

c) **Hard-to-clean contact areas**

Hard-to-clean areas have a high potential to harbor bacterial growth and should be tested regularly.

*Examples:* hoses, O-rings, nozzles, areas with irregularly shaped surfaces, corners, grooves, cracks and joints

The suggested sampling technique for a hose nozzle is to swab around the outer nozzle threads twice, and then swab around the inside lip once. Apply firm pressure while swabbing.
2.2.3 Diagram of typical production floor control points

Below is an example of a typical food processor’s production floor plan. Direct contact surfaces are indicated with a diamond, and indirect ones with a circle.
2.2.4 Monitoring individual control points

Once control points have been identified, this information should be entered into the Sure Trend software. In Sure Trend software, you can assign each control point a program (PROG) number that will correspond to a PROG number on the EnSURE & SystemSURE Plus luminometers.* Program locations can also be identified by name and “group” in SureTrend. Test results from individual control points can now be uploaded from the luminometer to the Sure Trend database for recording, tracking and analysis purposes.

Below is an example of a spreadsheet created with the SureTrend software. The program (PROG) numbers correspond to the control points illustrated in the Diagram of typical production floor test locations shown on the previous page.

<table>
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<th>Prog #</th>
<th>Location</th>
<th>Group</th>
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<th>Upper</th>
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<tr>
<td>2</td>
<td>Line A</td>
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</tr>
<tr>
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</tr>
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<td>6</td>
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</tr>
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<tr>
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<td>Line A</td>
<td>Blades</td>
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<td>Line A</td>
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<td>Hand Washing Station</td>
<td>On/Off Mechanism</td>
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</tbody>
</table>

For further information about the installation and use of Sure Trend software, see Managing and tracking your data with SureTrend Software in section 2.5.

*You may wish to create a legend that matches PROG numbers with location for easy reference during sampling. This legend can be attached to the key ring holder on the luminometer.
2.3 Determining ATP threshold levels for your facility

Setting correct *Pass*, *Caution* and *Fail* levels is a fundamental part of running a successful ATP hygiene monitoring program. These threshold levels may vary depending on the type of product being manufactured or processed as well as the surface or liquid being checked. However, the formulas used to determine *Pass*, *Caution* and *Fail* levels are always the same.

There are two different standards by which to set up *Pass/Fail* levels: *Routine Cleaning* and *Vigorous Cleaning*. The Vigorous Cleaning setup option is used by facilities requiring extremely high hygiene standards and/or which have easy-to-clean surfaces. Both methods produce similar results with slight variation.

Scigiene also provides general recommended threshold levels, which meet most manufacturers' hygiene standards. Examples of some typical threshold guidelines specific to the food and beverage industry can also be found in *Typical ATP threshold guidelines*, in Appendix A.

2.3.1 Options for setting up ATP threshold levels

a) Routine Cleaning

1. Identify control points in your facility.
2. Clean product surfaces to the level that daily sanitation programs should achieve.
3. Conduct an ATP test at each location. Take 5-10 replicate tests at each location over the course of several days.
4. **To determine the Pass limit:** Calculate the average RLU for each location based on the 5-10 test results. To do this, add all test results, and then divide the sum by the number of tests taken. The resulting number is your average RLU. This number is your *Pass* limit.
5. **To determine the Fail limit:** Multiply the *Pass* limit by 3. The resulting number is your *Fail* limit.
6. **To determine the Caution limit***: The area between the *Pass* and *Fail* thresholds is the *Caution* zone.

**NOTE:** In some cases, test results from individual control points within a facility or specific area are consistently within a similar range of each other. In this case, the user may choose to apply a single *Pass/Caution/ Fail* standard to all control points within that area or facility.
b) Vigorous Cleaning

1. Identify control points in your facility.
2. Clean all surfaces thoroughly (2 or more times) to achieve the maximum level of cleanliness possible. This may include a total production line disassembly.
3. Conduct an ATP test at each location. Take 5-10 replicate tests at each location over the course of several days.
4. **To determine the Pass limit:** Calculate the average RLU for each location based on the 5-10 test results. This average RLU is considered to be the Pass level.
5. **To determine the Fail limit:** Multiply the Pass limit by 2 for easy-to-clean areas and by 3 for harder-to-clean areas.
6. **To determine the Caution limit**: The area between the Pass and Fail thresholds is the Caution zone.

The following table uses example data to illustrate average RLU and Pass/ Caution/Fail thresholds:

<table>
<thead>
<tr>
<th>Test</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>Average RLU</th>
<th>Pass</th>
<th>Caution</th>
<th>Fail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine cleaning</td>
<td>10</td>
<td>15</td>
<td>8</td>
<td>19</td>
<td>10</td>
<td>13</td>
<td>17</td>
<td>14</td>
<td>15</td>
<td>11</td>
<td>13.2</td>
<td>13</td>
<td>14 - 35</td>
<td>36</td>
</tr>
<tr>
<td>Vigorous cleaning</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2.1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>(easy-to-clean)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vigorous cleaning</td>
<td>6</td>
<td>3</td>
<td>8</td>
<td>4</td>
<td>7</td>
<td>5</td>
<td>8</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5.4</td>
<td>5</td>
<td>6 - 14</td>
<td>15</td>
</tr>
<tr>
<td>(hard-to-clean)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** Different surfaces have different levels of risk, and therefore may require different thresholds. For example, porous plastic or rubber surfaces may be more difficult to clean than stainless steel surfaces, and therefore produce higher ATP test results. In this case, the user may choose to: 1) set thresholds higher for those harder-to-clean areas; or 2) intensify cleaning to bring ATP test results on those surfaces in line with other control points. This is up to the user.

* **NOTE:** The Caution zone is useful for trend analysis and providing early warnings. However, users may opt to forgo the Caution zone and set the Fail limit 1 RLU more than the Pass limit. Any result over the Pass limit is then considered a failed result.
2.3.2 General Pass/Caution/Fail levels

For facilities that opt not to set their own ATP thresholds, Hygiena offers two general guidelines for threshold levels based on the type of surface being tested. These are common levels used for ATP hygiene monitoring, and are based on plate count and ATP correlation studies. They may or may not suit the standards at every facility.

To determine independent ATP thresholds follow the “Routine Cleaning” or “Vigorous Cleaning” options described above.

a) Easy-to-clean surfaces

<table>
<thead>
<tr>
<th>RLU</th>
<th>Easy-to-Clean Surfaces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pass</td>
</tr>
<tr>
<td>EnSure &amp; Supersnap</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>SystemSure Plus &amp; Supersnap</td>
<td>&lt; 50</td>
</tr>
<tr>
<td>EnSure &amp; Ultrasnap</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>SystemSure Plus &amp; Ultrasnap</td>
<td>&lt; 10</td>
</tr>
</tbody>
</table>

b) Hard-to-clean surfaces

<table>
<thead>
<tr>
<th>RLU</th>
<th>Easy-to-Clean Surfaces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pass</td>
</tr>
<tr>
<td>EnSure &amp; Supersnap</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>SystemSure Plus &amp; Supersnap</td>
<td>&lt; 50</td>
</tr>
<tr>
<td>EnSure &amp; Ultrasnap</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>SystemSure Plus &amp; Ultrasnap</td>
<td>&lt; 10</td>
</tr>
</tbody>
</table>
2.3.3 Storing and viewing threshold data

Once threshold levels are established, they can be programmed into SureTrend, and then uploaded from the software to the EnSURE or SystemSURE Plus luminometers. For information on how to do this, see *Managing and tracking your data with SureTrend Software*, in section 2.5. Threshold data can also be manually entered into the luminometer (consult your *SystemSURE Plus User Manual* for details).

Once thresholds have been entered into the luminometer, upper (▲) and lower (▼) threshold limits are displayed numerically on the screen for each PROG location.

After completing an ATP test, *Pass/Caution/Fail* results will display on the EnSURE or SystemSURE Plus luminometers as follows:

✔️ *(Pass)* - for any RLU reading that is less than or equal to the *Pass* limit. A pass result indicates the surface is clean.

❗️ *(Caution)* - for any RLU reading that is greater than the *Pass* limit, but less than the *Fail* limit. A caution result indicates the surface may not have been adequately cleaned.

❌ *(Fail)* - for any RLU reading that is equal to or greater than the *Fail* limit. A fail result indicates the surface is dirty or contaminated.

For information on what steps should be taken after obtaining *Pass, Caution* or *Fail* results, see *Corrective action procedures* in section 2.5.
2.4 When to perform ATP testing

Once control points have been established and ATP threshold levels have been set, you should develop a sampling frequency plan.

Sampling frequency should be based on the level of contamination risk associated with the control point being tested. Factors determining the level of risk include:

- surface robustness and susceptibility to contamination
- degree of difficulty to clean
- frequency of contact with product
- for food processors, number of types of food being processed on a machine, which increases the potential for cross-contamination and allergens

Critical (high-risk) control points should be tested on a daily basis, after each cleaning. You may also choose to test after product line changes, after daily/employee shift changes, and or any time prior to start-up. Lower-risk control points may not need to be tested as frequently.

NOTE: ATP testing should be done prior to the application of a sanitizer if possible.

Swabbing should be done after cleaning a surface, but prior to the application of a sanitizer. By doing it in this order, you can remove any residue detected by the EnSURE or SystemSURE Plus with proper cleaning before applying sanitizer. The sanitizer is most effective when surfaces are free of all residue. This ensures microorganism removal when the sanitizer is used at its normal working strength.

Following this Clean-Test-Sanitize procedure prevents wasting time, money and chemicals on re-cleaning and re-sanitizing upon a failed result.

NOTE: Some sanitation procedures may require ATP testing be done after sanitizing, e.g. CIP (Clean-In-Place) systems. In such cases, allow the sanitizer to fully evaporate before ATP testing. For a list of sanitizers that could affect the ATP bioluminescence reaction, contact Scigiene.
2.5 Corrective action procedures

Implementing a corrective action plan is an essential part of the EnSURE & SystemSURE Plus ATP Detection Programs. Corrective action procedures provide clear instructions for what steps should be taken following Pass/Caution/Fail results.

Recommended corrective action procedures are as follows:

<table>
<thead>
<tr>
<th>ATP TEST RESULT</th>
<th>CORRECTIVE ACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>✔️ (Pass)</td>
<td>The control point surface has been adequately cleaned. Proceed to sanitizing.</td>
</tr>
<tr>
<td>! (Caution)</td>
<td>The control point surface may not have been adequately cleaned. You may choose to proceed to sanitizing, or re-clean and re-test. A control point with a Caution reading should be noted and monitored for future problems.</td>
</tr>
<tr>
<td>✗ (Fail)</td>
<td>The control point surface is dirty or contaminated, and must be cleaned again and re-tested until a Pass or Caution level is achieved. A control point with a Fail reading should be noted and monitored for future problems.</td>
</tr>
</tbody>
</table>

According to the needs of the facility, the user has the option of setting up a more extensive corrective action plan. For example, it may be decided that control points which produce Fail results should be re-tested until 3 consecutive days of Pass results are achieved. If the control point does not successfully achieve 3 consecutive days of Pass results, cleaning procedures or thresholds are required to be re-evaluated.

A flow chart on the next page illustrates general recommended cleaning and corrective action procedures.
Flow Chart: Suggested Cleaning, Test and Correction Action Procedures

1. Check detergent concentration is within specification
   - Proceed with cleaning Control Points (CP)

2. Standard cleaning routine
   - Re-clean work area
   - Visibly clean
   - Perform rapid ATP hygiene confirmation
   - SystemSURE Plus Detection Test
     - PASS: Continue on to sanitizing OR No further action required
     - CAUTION: Re-clean & re-test CP OR Continue
     - FAIL: Repeat ATP test for confirmation. If CP fails again, re-clean.

3. Sanitizing routine
   - Optional ATP test to confirm cleanliness
2.4 Continuous improvement

The EnSURE & SystemSURE Plus ATP Detection Programs are designed to aid organizations striving for continuous improvement of standards. Continuous improvement ensures excellence in product quality, while reducing inefficiencies, avoiding recalls, and showing due diligence and compliance to auditors.

Analysis of ATP test result trends is key to the evaluation and ongoing improvement of the sanitation system in place. Using the SureTrend Data Analysis software, you can monitor and assess ATP test results, perform trend analysis, identify trouble zones, correct improper cleaning procedures and eliminate risk.

If trends show high numbers of Caution and Fail results in ATP testing, SSOPs should be reviewed by experts for ways to improve protocols. If low numbers of Caution and Fail results are obtained, Pass/Fail thresholds should be reviewed and lowered to maintain high standards and ensure continuous improvement.

Pass, Caution and Fail thresholds should also be re-evaluated every year and whenever procedural changes are made. As sanitation procedures become more efficient and effective, thresholds should be adjusted to ensure your facility is operating to its maximum potential.
# SECTION 3: Further help

## 3.1 Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>You are ready to insert the activated Supersnap or Ultrasnap device into the luminometer, but the screen on the luminometer says CAL, with numbers counting down.</td>
<td>The EnSURE &amp; SystemSURE Plus luminometers automatically performs a 60-second self-calibration (CAL) upon startup. Always be sure to turn the luminometer on and wait for self-calibration to finish BEFORE activating the Supersnap or Ultrasnap device.</td>
</tr>
<tr>
<td>Calibration Control Rod Kit results are consistently outside of the recommended range.</td>
<td>There may be a problem with your luminometer. Contact Scigenie Corporation.</td>
</tr>
<tr>
<td>Positive Control Kit results are consistently outside of the recommended range.</td>
<td>There may be a problem with your swab devices or the luminometer. Contact Scigenie Corporation.</td>
</tr>
<tr>
<td>ATP test results are inconsistent. There is a large discrepancy between readings at the same location.</td>
<td>Review cleaning and testing procedures. Some factors affecting consistency of results include poor or inconsistent adherence to standard cleaning procedures, faulty detergent concentration, or inconsistent sampling technique.</td>
</tr>
</tbody>
</table>
3.2 Glossary

ATP (adenosine triphosphate)
ATP is a chemical compound used by all living organisms to fuel metabolic processes such as muscle function and reproduction. ATP is found in living organisms (including bacteria and other microorganisms) as well as once-living material (such as food). Detection of ATP on a surface indicates the presence of either microbial contamination or food residue that has the potential to support microbe growth.

Bioluminescence
Bioluminescence is a chemical reaction that produces light when ATP comes into contact with the enzyme luciferase. The Ultrasnap Sample Testing Device uses bioluminescent technology (combining ATP with luciferase) to produce a light output that can be detected and measured by the SystemSURE Plus luminometer. Light output during the Ultrasnap reaction is directly proportional to the amount of ATP on the sample.

RLU (Relative Light Units)
RLU is the unit of measurement for light output during a bioluminescent reaction. The SystemSURE Plus luminometer expresses ATP test results in RLU. RLU numbers are directly proportional to the amount of ATP on the test sample, therefore a high RLU indicates a large amount of ATP present, which in turn indicates a high degree of contamination. Low RLU indicates low levels of ATP and low level of contamination.

Biofilm
Biofilms occur when microorganisms work together as a population and secrete a sticky polymer to form a solid matrix attached to a surface. Once a biofilm is established it is very difficult to eliminate. Biofilms can cause poor product quality and/or lost product due to contamination.

HACCP (Hazard Analysis and Critical Control Points)
HACCP is a widely accepted systematic approach to the identification, evaluation and control of significant food safety hazards in the food manufacturing and processing industries.

SSOP (Sanitation Standard Operating Procedures)
Sanitation procedures in food production plants. They are considered a basic requirement in a HACCP program.

Control Points (CP)
Control points are direct and indirect contact areas where potential contamination hazards can be identified, controlled and monitored.
3.3 Appendix A: Typical ATP threshold guidelines

The following guidelines are typical of those found in the food and beverage sectors and are based on stainless steel surfaces*. Other surfaces may be more difficult to clean and consequently may require a higher Pass/Fail level to be set.

These guidelines represent those typically used in the sectors listed. These should only be used for guidance in establishing *Pass, Caution and Fail* thresholds for similar surfaces and products.

When using the SystemSURE Plus luminometer with the Ultrasnap swabs (ATP2020), 1fmol ATP = 1RLU. You may use the RLU thresholds listed below without any change.

If using the EnSURE luminometer with the Ultrasnap swabs (ATP2020), 0.5fmol ATP = 1RLU. This new EnSURE luminometer is now 2X more sensitive. Therefore, you would multiply the below RLU values by 2 to determine your pass/fail thresholds.

If using the SystemSURE Plus with the Supersnap swabs (ATP3000), 0.2fmol ATP = 1RLU. The Supersnap swabs are 5X more sensitive when used in these 2 luminometers. Therefore, you would multiply the below RLU values by 5 to determine your pass/fail thresholds.

If using the EnSURE luminometer with the Supersnap swabs (ATP3000), 0.1fmol ATP = 1RLU. The Supersnap swabs are 10X more sensitive when used in this new luminometer. Therefore, you would multiply the below RLU threshold values by 10 to determine your pass/fail thresholds.

---

**High Risk Product Surfaces/CIP (Clean-In-Place) systems**

<table>
<thead>
<tr>
<th>PRODUCTION ENVIRONMENTS</th>
<th>PASS</th>
<th>CAUTION</th>
<th>FAIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy (milk, cream products)</td>
<td>&lt; 15</td>
<td>16 – 29</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>Juice products</td>
<td>&lt; 15</td>
<td>16 – 29</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>Water bottling</td>
<td>&lt; 15</td>
<td>16 – 29</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>Brewing plant and equipment</td>
<td>&lt; 15</td>
<td>16 – 29</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>CIP (Clean-in-Place) Systems</td>
<td>&lt; 5</td>
<td>6 – 9</td>
<td>&gt; 10</td>
</tr>
</tbody>
</table>
Typical Product Surfaces

<table>
<thead>
<tr>
<th>PRODUCTION ENVIRONMENTS</th>
<th>PASS</th>
<th>CAUTION</th>
<th>FAIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw meat Abattoirs</td>
<td>&lt; 100</td>
<td>101 - 199</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>Butchery</td>
<td>&lt; 50</td>
<td>51 - 99</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Cooked meat products</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Meats &amp; all product w/meat)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low risk</td>
<td>&lt; 50</td>
<td>51 - 99</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>High risk</td>
<td>&lt; 25</td>
<td>26 - 49</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>Fish products</td>
<td>&lt; 30</td>
<td>31 - 59</td>
<td>&gt; 60</td>
</tr>
<tr>
<td>Shellfish</td>
<td>&lt; 100</td>
<td>101 - 189</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>Cheese processing</td>
<td>&lt; 25</td>
<td>26 - 49</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>General food processors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low risk</td>
<td>&lt; 30</td>
<td>31 - 49</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>High risk</td>
<td>&lt; 10</td>
<td>11 - 29</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>Vegetable processing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low risk</td>
<td>&lt; 50</td>
<td>51 - 74</td>
<td>&gt; 75</td>
</tr>
<tr>
<td>High risk</td>
<td>&lt; 10</td>
<td>11 - 29</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>Baked products</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low risk</td>
<td>&lt; 30</td>
<td>31 - 49</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>High risk</td>
<td>&lt; 10</td>
<td>11 - 29</td>
<td>&gt; 30</td>
</tr>
</tbody>
</table>

*For recommended thresholds in other environments, such as food service, hospitality, government, education, pharmaceutical and healthcare, contact Scigiene Corporation.
3.4 Other Scigenie products

**Microsnap** The Micro-Snap Enrichment Swab is a rapid test for enumeration of Coliforms and E.coli. This is a 2 step procedure and results are obtained within 4 to 7 hours. Swab a 10 x 10 cm area or prepare a suspension and transfer to the Enrichment swab as per instructions and incubate for 7 hours. After incubation, transfer 100ul from the Enrichment Swab to the fill line on the Detection Device (Part#: ATP-CC100 for Coliforms or ATP-EC100 for E.coli and Enrichment swab ATP-ES100).

**Aquasnap** A user-friendly, self-contained ATP water sampling device used with the EnSURE, SystemSURE Plus and Pi-102 luminometers (Part#: ATP-150-total ATP or ATP-151-free ATP)

**PRO-Clean** Quickly and accurately monitors the cleanliness of surfaces to ensure product safety and quality by detecting protein residues left on a surface after cleaning. Simply swab a surface, release the reagent and if protein residue is present the reagent will turn purple. The color change provides a semi-quantitative measure of the surface cleanliness. Detects down to 20 ug of protein and requires no instrumentation (Part#: ATP-120).

**Allersnap** Detects down to 1ug of protein residues left on a surface after cleaning and therefore suitable for allergen detection. Simply swab a surface, release the reagent, incubate, and if protein residue is present the reagent will turn purple. Enhanced sensitivity by incubating at 55°C for 15 minutes or 37°C for 30 minutes. (Part#: ATP-121).

**SnapShot** A universal ATP swab designed for luminometers from other manufacturers. No need to change threshold settings on the luminometer being used. Made with unique liquid-stable reagents to yield more accurate and consistent results (Part#: ATP110+).

**SpotCheck** A surface hygiene swab that detects glucose when food residue is present. Requires no instrumentation. The self-contained reagent turns from clear to green (Part#: ATP130).

**SpotCheck Plus** SpotCheck Plus detects lactose and glucose residue in under 60 seconds. Presence of either substance is indicated by a color change of a self-contained reagent. Requires no instrumentation (Part#: ATP140).

**Pi-102** An affordable multifunctional luminometer using traditional photomultiplier tube technology. For use with cuvettes or all-in-one reagent devices such as Supersnap, Ultrasnap, Aquasnap or Snapshot. Suitable for bioluminescence and chemiluminescence assays (Part#: ATP225).

**Q-Swab** Increase productivity and efficiency while reducing lab costs by using Q-swab for your environmental monitoring needs. Q-swab comes with multiple 1ml buffer formulations for optimal recovery of bacteria. No need for broth preparation. Available in Letheen Broth, Buffered Peptone Water, Stuart’s Medium, and Amie’s Medium (Part#: ATP1200-1400).
**QD-Loop** All-in-one sterile system for accurate and convenient volumetric dilutions. Designed with patented Snap-Valve technology. Devices are calibrated for 1/10, 1/100 or 1/1000 dilutions (Part#: ATPQD0010-1000).

We also supply other microbiology tools including sponges, sample bags, and equipment for all of your environmental sampling needs.


### 3.5 Contact Scigiene Corporation

For any questions about the Pi102, EnSURE and SystemSURE Plus ATP Detection Programs, or for more information on other temperature & hygiene monitoring products, please contact Scigiene at:

[Scigiene Corporation](http://www.scigiene.com)