MicroSnap™ – Total

Part Numbers:
• MicroSnap™ Total Enrichment Device (Part # ATP-ENT200)
• MicroSnap™ Total Detection Device (Part # ATP-APC200)

Description / Intended Use:
MicroSnap™ Total is a rapid bioluminogenic test for detection and enumeration of total population of viable aerobic bacteria in a sample providing results in 7 hours. MicroSnap™ Total consists of an Incubation Device containing proprietary growth media and a Detection Device containing bioluminogenic reagents in which biomarkers produced by bacteria are measured using a small portable luminometer.

The two-step test procedure requires a short incubation period facilitating growth of bacteria followed by a detection step. During incubation, bacterial numbers increase, and sample interference is reduced. As bacteria grow, they use up available food resources in the media and generate biomarkers. The greater the number of bacteria in the sample, the higher the biomarker concentration, and the greater output of light. An aliquot of enriched sample is transferred to the Detection Device, activated, mixed, and measured in a luminometer. Light output is directly proportional to initial starting concentration of bacterial contamination in pre-enriched samples.

Intended User:
Laboratory personnel trained in standard microbiological practices are qualified to use MicroSnap™ Total.

Applicability:
MicroSnap™ Total is applicable for enumeration of metabolically viable aerobic bacteria from environmental surfaces, product samples, and liquids. The method was validated through the AOAC Performance Tested Methods (PTM) Program for a wide range of foods including major food groups such as meat, dairy, and vegetables. . Refer to AOAC-RI PTM Certificate #031501 for details.

Limitations:
The MicroSnap™ Total method relies on the measurement of ATP as the prime metric. MicroSnap™ Total has not been evaluated with all possible matrices. See User Responsibility.

It is important that samples are brought to ambient temperature (20-25°C) prior to use with MicroSnap™. Samples that are not brought to ambient temperature before incubation (taken directly from refrigeration ~4°C) will under-detect due to time lag in reaching 30°C incubation temperature.

It is important all media or diluents used with MicroSnap™ Total are sterile. Inhibitors in media and diluents are the prime reason for most unsuccessful detections. Scigiene recommends the diluents listed below.

Required Materials To Purchase From Scigiene™(Not Provided):
- EnSURE™ Touch (Part#: ATP-207) or EnSURE™ luminometer (Part#: ATP-206)
- Incubator at 30°C ± 0.5°C

Materials Required When Testing Product Samples:
- Sample bags
- Homogenizing equipment
- Pipette and tips for 1 mL
- Product Sample Diluents:
    - Buffered Peptone Water
    - Maximum Recovery Diluent
    - Butterfields
    - Sterile Water

Test Procedure:

<u>Step 1: Incubation</u>
1. Incubation procedure is described below and is also shown in Step 1 diagrams.
2. Allow MicroSnap™ Total Incubation Device to equilibrate to room temperature (10 minutes at 20-25°C). Collect sample and transfer aliquot into MicroSnap™ Incubation Device without touching the swab or inside of sample device with fingers.
3. Samples Preparations Include:

a) Surfaces Samples – Utilize pre-moistened Incubation Device to sample a 4 x 4 inches (10 x 10 cm) square area. For irregular surfaces, ensure swabbing technique remains consistent for each test and swab a large enough area to collect a representative sample.

b) Important swabbing technique tips:
   i. Rotate swab while collecting sample to maximize sample collection on swab tip.
   ii. Apply sufficient pressure to create flex in swab shaft.
   iii. Swab in a crisscross pattern vertically, horizontally, and in both diagonal directions.

c) Liquid Samples – Transfer 1mL of liquid or water samples added directly to Incubation Device.

d) Product Samples – 1 mL of appropriate suspension, e.g., 10% w/v (weight / volume) food homogenate added directly to Incubation Device. Food homogenate should be prepared by weighing out 10 g or 50 g of food matrix and adding it to a stomacher bag containing 90 mL or 450 mL diluent (Note: Maximum Recovery Diluent was validated in the AOAC PTM study). For unknown sample contamination, dilutions below 10% should be produced in more diluent by adding 10 mL of 10% into 90 mL of fresh diluent and repeating for 1% and 0.1%. If replicate samples are required, then another 10 g or 50 g should be removed from the bulk matrix and the dilutions series repeated. Replication can be achieved by drawing multiple 1 mL aliquots from either the 10%, 1%, or 0.1% dilutions depending on RLUs achieved. Note: When performing comparison testing, sample assays must be started within 10 minutes for comparable results between methods. Samples taken can be stored prior to use at 4°C for up to 2 days but must be equilibrated back to ambient temperature before samples are run on MicroSnap™ or any equivalent methods.

4. Re-attach swab back into to swab tube. Device should look the same as it did when first pulled from the bag.
5. Activate Incubation Device by holding swab tube firmly and using thumb and forefinger to break Snap-Valve by bending bulb forward and backward.
6. Separate bulb and swab tube about 1 – 2 inches from each other, relieving internal pressure, and squeeze bulb to flush all media to bottom of swab tube. Ensure most of broth is in bottom of swab tube.

7. Re-attach swab back on to swab tube firmly to seal device.
8. Shake tube gently to mix sample with broth.
9. Incubate at 30 ± 0.5°C for 7 hours ± 10 minutes.

Step 2: Detection

Detection procedure is described below and is also shown as Step 2 in the diagram below. Before beginning Step 2, turn on your luminometer. If you have programmed your MicroSnap sample, open to test screen of the sample you'd like to test.

1. Allow MicroSnap™ Total Detection Device to equilibrate to room temperature (10 minutes at 20-25°C).
   a) Shake test device by either tapping on palm of hand 5 times, or forcefully flicking in a downward motion once.
   b) This will bring extractant liquid dispersed in tube to bottom of tube.
   c) Extractant is necessary to facilitate mixing of enriched sample with solution in tube.
2. Transfer enriched sample from Incubation Device to Detection Device.
   a) Aseptically transfer 0.1 mL of enriched solution by utilizing the built-in dropper tip of the Incubation Device.
      i. Squeeze and release bulb of Incubation Device to mix and draw sample into dropper tip.
      ii. Aseptically open Incubation Device and open Detection Device by twisting and pulling to remove bulb. Set aside. Insert Incubation Device swab tip 1 inch or 3 cm into top of Detection Device tube and lightly squeeze Incubation Device bulb to trickle 3 drops of enriched sample into tube until volume reaches fill line marked on bottom of Detection Device tube. Avoid adding excess sample above fill line, as this can increase variation of test results.
      iii. Remaining enriched sample can be returned to Incubation Device for additional testing. Reassemble Incubation Device to original state and return device to incubator. Note: When testing replicates from same enriched sample, all replicates must be performed within 10 minutes to obtain comparable results.
3. Activate Detection Device by holding swab tube firmly and using thumb and forefinger to break Snap-Valve by bending bulb forward and backward. Squeeze bulb 3 times to release all liquid to bottom of swab tube.
4. Shake gently for 2 seconds to mix.
5. Immediately insert whole device into luminometer; close lid and holding unit upright, press the button to initiate the measurement. Results will appear after 10 or 15 seconds, depending on the instrument you're using.
6. EnSURE™ Touch (Part#: ATP-207) gives results in 10 seconds. Results are shown in CFU. MicroSnap™ samples can be programed directly on the luminometer or by using SureTrend™ Cloud.
7. EnSURE™ (Part#: ATP-206) result will be displayed in RLU (Relative Light Units) in 15 seconds. Use SureTrend™ to program MicroSnap™ samples and set RLU thresholds on the EnSURE™ to correspond with required CFU (colony forming units) limits. Refer to "Interpretation of Results" below for correlation.

Potential Limit of Detection:
Limit of detection is the lowest level of viable aerobic bacteria that can be detected above a food matrix background when the assay is performed correctly and efficiently. The sensitivity increases as incubation time increases. At 7 hours the detection level approaches 10 to 100 CFU per mL of Incubation Media; at 8 hours this lowers closer to 1 CFU per mL. However, some organisms which are slower to grow or recover from stress, could take longer to reach 1 CFU. At 7 hours, the dynamic range of MicroSnap™ Total in the EnSURE™ luminometer is proportional to the actual range of RLU feasible in the EnSURE™ instrument. A lower limit of 10 RLU (<10 CFU/mL at 7 hours) is based on the natural background of sterile foods tested (RLU mean plus 6 standard deviations).

Table 1: Potential Dynamic Range at 7 hours Incubation

Sample Type	CFU Range
Surface	10-10,000 (CFU/swab)
1mL Liquid	10-10,000 (CFU/mL)
10% w/v Suspension of Solid	100-50,000 (CFU/g)

For samples where contamination is outside ranges detailed in Table 1, the following serial dilutions must be made in order to be read on the luminometer:

- 1% suspension will give a range of 1,000 – 500,000 CFU
- 0.1% suspension will give a range of 10,000 – 5,000,000 CFU
- Note: When testing multiple serial dilutions, all dilutions must be prepared and tested simultaneously to obtain linear results.

AOAC Performance Tested Methods Validation:

Table 2: AOAC PTM Validation Correlation to ISO Method for Various Food Matrixes

Food Matrix	Correlation to ISO Method (R2)
Raw Ground Beef	0.771*
Raw Chicken	0.969
Lettuce	0.948
Cream cakes	0.987
Raw Milk	0.990

EnSURE Touch Correlation to ISO Method R2 equals 0.956

Food matrixes were tested in their natural state; no spiking of bacteria was performed, and all samples rendered some form of countable range. Hence, the use of true negatives is difficult to perform with real food samples due to bacteria always being present even at low levels. The use of a lower limit of detection of 100 CFU per gram is acceptable when using MicroSnap™ at 7 hours incubation.

The method was shown to have good correlation with International Organization for Standardization (ISO) method, ISO 4833:2003, *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of microorganisms – Colony-count technique at 30 °C (ISO 4833)* (3) reference method for enumeration of total viable count (TVC).

Interpretation of Results:

For situations in which results are displayed in Relative Light Units (RLUs), the numerical output is proportional to ATP content extracted from growing viable bacteria at the time of testing. This ATP concentration is in turn proportional to the starting bacterial inoculums expressed as Colony Forming Units (CFU). Table 3 shows the equivalent CFU values for RLU measurements for 7 hours incubation at 30°C only. Data was generated from a variety of foods tested in internal and AOAC validation studies. EnSURE™ Touch makes this correlation easier because the software because its software does conversion for you, using the data generated from the AOAC Validation Studies as well as additional internal testing.

Table 3: Typical Correlation Between RLU and CFU at 30°C for EnSURE

RLU (EnSURE)	Equivalent CFU	
	Direct sample e.g., 1mL liquid (or surface swab)	Typical 10% suspension of solid sample
<10	<10	<100/g
<20	<20	<200/g
<30	<30	<300/g
<50	<50	<500/g
<100	<100	<1,000/g
<1000	<1000	<10,000/g
>5,000	TNTC	TNTC

Where several dilutions are prepared and tested for samples with unknown contamination, the CFU/ g or mL is calculated by multiplying the CFU result by the corresponding dilution factor. On EnSURE Touch, the dilution factor is included in the calculation, so you do not need to do additional calculations.

Calibration & Controls:
It is advisable to run positive and negative controls according to Good Laboratory Practices. Scigiene offers the following calibration verification devices:

- Calibration Control Kit (Part #: ATP4003-CT-E)
- CalCheck (Part #: ATP-CAL)

Sample Effects:
Samples Containing Natural Nucleotides:

- Depending on nucleotide levels in some sample types (e.g., some leafy greens) bacterial enumeration can be influenced or misinterpreted.
    - Prior to protocol implementation, check background levels by performing Detection Step 2 pre- and post-incubation
    - Dependent on results, threshold levels can be adjusted to accommodate background levels

Contact Scigiene™ for additional protocol or matrix support
Thick or Opaque/Dark Samples:

- Detection with the luminometer could be affected by the thickness or darkness of samples such as undiluted milk due to a blanching effect.
    - Prior to protocol implementation, direct test samples to observe any detection issues.
    - Dependent on results, a 1:10 dilution can be applied to reduce matrix effect during detection.

Storage & Shelf Life:

Store at 2 – 8°C. Devices have a shelf life of 12 months from the date of manufacturing. Check expiration date on label.

Disposal:

Disinfect before disposal. Components of MicroSnap™ devices do not pose any health risk when used correctly. Used devices that confirm positive results may be biohazardous and should be disposed of safely in compliance with Good Laboratory Practice and Health and Safety regulations. Disinfect before disposal. MicroSnap™ devices can be disinfected by autoclaving or by soaking in 20% bleach for 1 hour. Then, they can be placed in the trash. Alternatively, MicroSnap™ devices may be discarded at a biohazard waste disposal facility.

Safety & Precautions:

1. MicroSnap™ device components do not pose any health risk when used correctly. Used devices confirming positive results may be a biohazard and should be disposed of safely in compliance with Good Laboratory Practice and Health and Safety Regulations (see disposal instructions above).

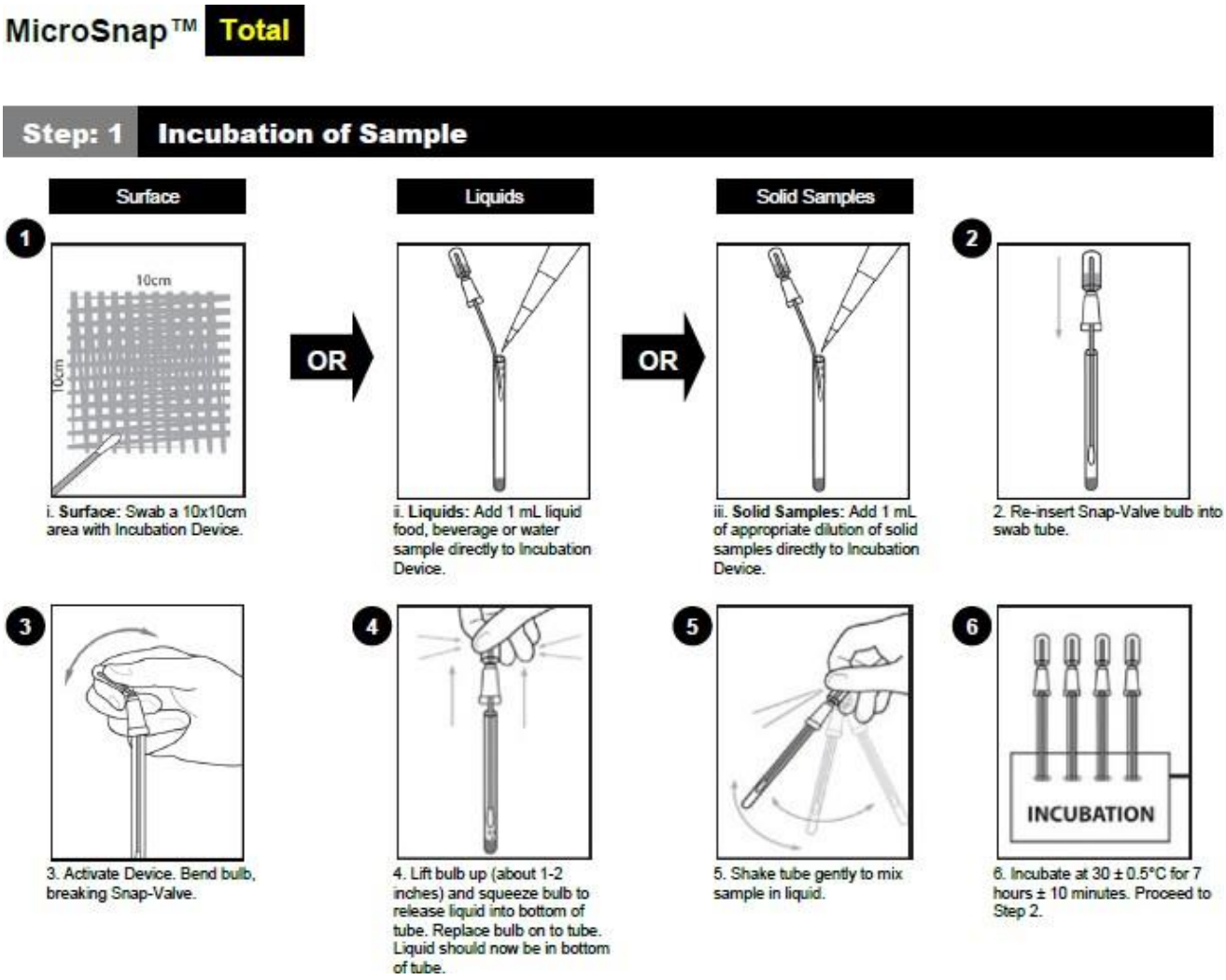
2. Devices are designed for a single use. Do not reuse.
3. Do not use devices after expiration date.
4. Sampling should be done aseptically to avoid cross contamination.
5. Verify proper incubation temperature and time for the test application. In most cases, this will be 6- or 8-hours incubation as specified in the above instructions, unless you've been directed otherwise by Scigiene for custom applications which require different incubation times or temperatures.
6. When testing multiple serial dilutions, all dilutions must be prepared and tested simultaneously to obtain linear results.
7. Ensure proper sample dilution so that it can be read within luminometer's dynamic range.
8. Ensure proper incubation temperature and time for the test application.
9. When testing replicates from same enriched sample, all replicates must be performed within 10 minutes to obtain comparable results.
10. When performing comparison testing, sample assays must be started within 10 minutes for comparable results between methods.

Caution & User Responsibility:

1. MicroSnap™ devices have not been tested with all possible food products, food processes, testing protocols or with all possible microorganism strains.
2. Do not use this test for diagnosis of conditions in humans and animals.
3. No single culture medium will recover the same strain or enumerate a particular strain in the same way as another medium. Other external factors such as sampling method, testing protocol, and handling may influence recovery.
4. It is the user's responsibility when selecting a test method to evaluate a sufficient number of samples.
5. As with any culture medium, MicroSnap™ results do not constitute a guarantee of product quality.
6. Personnel must be trained in proper testing techniques and standard microbiological practices.

Scigiene Liability:

As with any culture medium, MicroSnap™ Total results do not constitute a guarantee of quality of food, beverage products or processes that are tested with these devices. Scigiene will not be liable to user or others for any loss or damage, whether direct or indirect, incidental, or consequential from use of these devices. If this product is proven to be defective, Scigiene's sole obligation will be to replace product, or at its discretion, refund the purchase price. Promptly notify Scigiene within 5 days of discovery of any suspected defect and return product to Scigiene. Please call Customer Service for a Returned Goods Authorization Number.

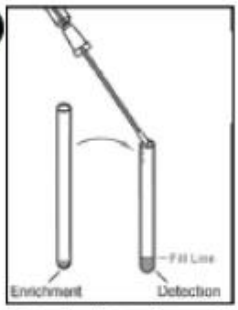


Step: 2 Detection / Measurement

1

1. Allow Detection Device to equilibrate to room temperature. Shake to bring liquid in tube to bottom of tube.

2



2. Aseptically transfer enriched sample from Incubation Device to Detection Device.

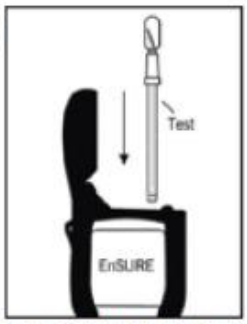
3

3. Activate Detection Device by breaking Snap-Valve. Squeeze bulb to release liquid into tube. Liquid should now be in bottom of tube.

4

4. After activation, shake tube gently to mix sample in liquid.

5a

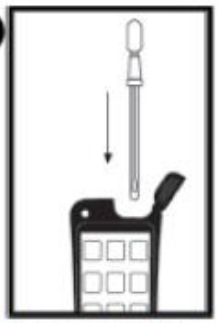


5a. For EnSURE™ Touch, skip to 5b. On EnSURE™, insert Detection Device and press "OK" to initiate measurement.

6a

6a. Record RLU results and refer to the Results Interpretation Table for the conversion.

5b



5b. On EnSURE™ Touch, open the MicroSnap™ application and select "Quick Test" if you are testing a surface and have not programmed your sample or select Samples if you have programed your sample . Press "Run Test."

6b

6b. EnSURE™ Touch automatically saves your results. To get the most value out of your EnSURE™ Touch, register and sync it wirelessly to SureTrend™ Cloud where you can generate meaningful reports and view datasets.

Scigiene

1295 Morningside Avenue, Unit 16-18
Scarborough, ON M1B 4Z4 Canada
Phone: 416-261-4865 Fax: 416-261-7879
www.scigiene.com