MicroSnap™ Total

Rapid Total Microbial Activity Test

Required Parts:
Step 1: MicroSnap Total Enrichment Device, MS1-TOTAL (QTY 100)
Step 2: MicroSnap Total Detection Device, MS2-TOTAL (QTY 100)

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 031501

hygiena

Description:

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 Enrichment 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 in enrichment media, bacteria numbers increase and sample interference is reduced. As bacteria grow they use up available food resources in media and generate biomarkers. The greater the number of bacteria in the sample, the higher the biomarker concentration, and the more light output. An aliquot of enriched sample is transferred to the Detection Device, activated, mixed and measured in a luminometer. Light output is directly proportional to concentration of bacteria present.

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 foods, liquids and surfaces. The method was validated through the AOAC *Performance Tested Methods*^{SM} (PTM) Program for various foods. 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 products. See User Responsibility.

It is important samples are at ambient temperature (18- 25 °C) prior to use in MicroSnap. Incubations begun from samples directly removed from 4°C refrigeration 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 free of bioluminescence inhibitors. Inhibitors in media and diluents are the prime reason for most unsuccessful detections. Hygiena recommends diluents listed below.

Required Materials (Not Provided):

- Incubator at 30 ± 0.5 °C
- EnSURE luminometer (Part No.: ENSURE)

For product samples:

- Diluents
  - Buffered Peptone Water
  - Maximum Recovery Diluent
  - Butterfields
- Sample bags
- Homogenizing equipment
- Pipette and tips for 1mL

Directions:

Instructional Video: www.youtube.com/HygienaTV

Step 1: Enrichment

Enrichment procedure is described below and is also shown in Step 1 diagrams.

1. Collect sample and place in MicroSnap Total Enrichment Device (Part # MS1-TOTAL)
   Samples can be:
   i. Surface - Swab a 4 x 4 inch (10 x 10 cm) square area, or for irregular surfaces, as much of surface as possible to collect a representative sample.
   ii. Liquid - 1mL liquid food, beverage or water samples added directly to Enrichment Device.
   iii. Product - 1mL of appropriate suspension, e.g. 10% w/v (weight / volume) food homogenate added directly to Enrichment Device. Food homogenate should be prepared by weighing out 10g or 50g of food matrix and adding it to a stomacher bag containing 90mL or 450mL 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 10mL of 10% into 90mL of fresh diluent and repeating for 1% and 0.1%. If replicate samples are required then another 10g or 50g should be removed from the bulk matrix and the dilutions series repeated. Replication can be achieved by drawing multiple 1mL aliquots from either the 10%, 1%, 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 before samples are run on MicroSnap and any equivalent methods.
2. Re-attach swab back on to swab tube. Device should look the same as it did when first pulled from bag.
3. Activate Enrichment Device by holding swab tube firmly and using thumb and forefinger to break Snap-Valve by bending bulb forward and backward.
4. 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 enrichment broth is in bottom of swab tube.
5. Re-attach swab back on to swab tube firmly to seal device.
6. Shake tube gently to mix sample with enrichment broth.
7. Incubate at 30 ± 0.5 °C for 7 hours ± 10 minutes.

Step 2: Detection

Detection procedure is described below and is also shown in Step 2 diagrams. Before beginning Step 2, turn on EnSURE luminometer. If locations have been programmed, select location to be tested.

1. Allow MicroSnap Total Detection Device (Part # MS2-TOTAL) to equilibrate to room temperature (10 minutes at 22 – 26 °C).Shake test device by either tapping on palm of hand 5 times, or forcefully flicking in a downward motion once. This will bring extractant liquid to bottom of tube.
2. Transfer enriched sample from Enrichment Device to Detection Device. Enrichment Device swab can be used as a pipette for convenience.
   i. Squeeze and release Enrichment Device bulb to mix and draw sample into bulb.
   ii. Remove Enrichment Device swab from tube.

iii. Open Detection Device by twisting and pulling to remove bulb. Set aside.
   a. Insert Enrichment Device swab tip into top of Detection Device tube (approximately 1 inch or 3 cm) and lightly squeeze Enrichment Device bulb to trickle 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.

iv. Remaining enriched sample can be returned to Enrichment Device for additional testing. Reassemble Enrichment 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.

v. Reassemble Detection Device to original state.

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 to mix.
5. Immediately insert whole device into luminometer; close lid and holding unit upright, press "OK" button to initiate measurement. Results will appear after 15 second count down.
6. Result will be displayed in RLU (Relative Light Units). Set RLU thresholds on instrument to correspond with required CFU limits. Refer to "Interpretation of Results" below for correlation.

Limit of Detection:

Limit of detection is the lowest level of viable aerobic bacteria that can be detected above a food matrix background when assay is correctly and efficiently performed. The lowest level of bacteria that can be detected decreases as incubation time increases. At 7 hours the detection level approaches 10 CFU per mL of Enrichment Media; at 8 hours this lowers to 1 CFU per mL. The continuation of this stoichiometry is in line with the Baranyi-Roberts model of bacterial growth in foods. At 7 hours incubation time, the CFU to RLU relationship resembles a 1 to 1 or Y=X relationship. 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: 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 be 1,000 – 500,000 CFU
- 0.1% suspension will be 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 PTM Validation:

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

Food Matrix	Correlation to ISO Method (R^2)
Raw Ground Beef	0.771
Raw Chicken	0.969
Lettuce	0.948
Cream cakes	0.987
Raw Milk	0.990

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 TVC.

Interpretation of Results:

Results are displayed on the luminometer as 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.

Table 3: Correlation between RLU and CFU at 30 °C

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. A convenient Microsoft Excel® calculator is available from Hygiena for calculating RLU to CFU conversions.

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Calibration & Controls:

It is advisable to run positive and negative controls according to Good Laboratory Practices. Hygiena offers the following instrument control:

- Calibration Control Kit (Part # PCD4000)

Storage & Shelf Life:

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

Disposal:

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. Components of MicroSnap devices 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. Detection Device is designed for a single use. Do not reuse.
2. Do not use devices after expiration date.
3. Sampling should be done aseptically to avoid cross contamination.
4. Ensure sample dilution is within luminometer's dynamic range.
5. Ensure proper incubation temperature and time as described in Directions.
6. When testing multiple serial dilutions, all dilutions must be prepared and tested simultaneously to obtain linear results.
7. When testing replicates from same enriched sample, all replicates must be performed within 10 minutes to obtain comparable results.
8. When performing comparison testing, sample assays must be started within 10 minutes for comparable results between methods.

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. Personnel must be trained in proper testing techniques.

Hygiena 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. Hygiena 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, Hygiena's sole obligation will be to replace product, or at its discretion, refund the purchase price. Promptly notify Hygiena within 5 days of discovery of any suspected defect and return product to Hygiena. Please call Customer Service for a Returned Goods Authorization Number**.**

Contact information:

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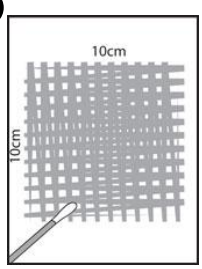
MicroSnap **Total**

Step: 1 Enrichment of Environmental Surface Swab, Liquid and Solid Samples

Surface

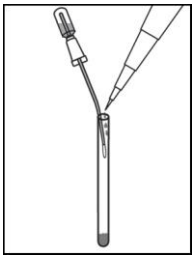
1



i. **Surface:** Swab a 10x10cm area with Enrichment Device.

OR

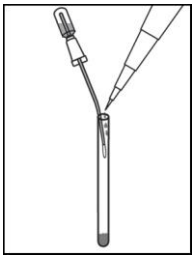
Liquids



ii. **Liquids:** Add 1mL liquid food, beverage or water sample directly to Enrichment Device.

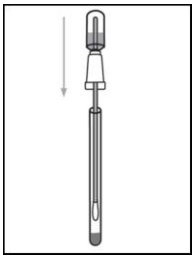
OR

Solid Samples



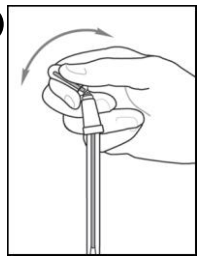
iii. **Solid Samples:** Add 1mL of appropriate dilution of solid samples directly to Enrichment Device.

2



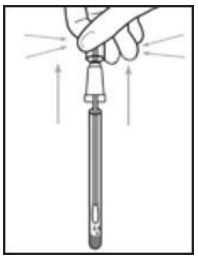
2. Re-insert Snap-Valve bulb into swab tube.

3



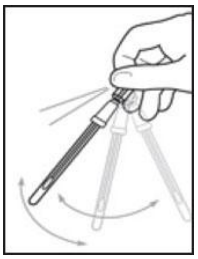
3. Activate Device. Bend bulb, breaking Snap-Valve.

4



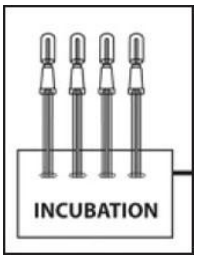
4. Lift bulb up (about 1 – 2 inches) and squeeze bulb to release liquid into bottom of tube. Replace bulb on to tube. Liquid should now be in bottom of tube.

5



5. Shake tube gently to mix sample in liquid.

6



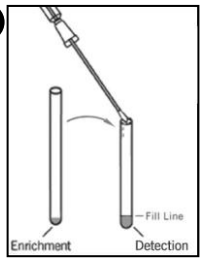
6. Incubate at 30 ± 0.5 °C for 7 hours ± 10 minutes. Proceed to Step 2.

Step: 2 Detection / Measurement

1

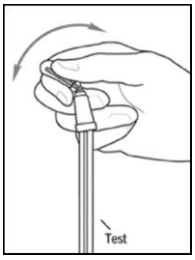
1. Allow Detection Device to equilibrate to room temperature. Shake to bring liquid in tube to bottom of tube.

2



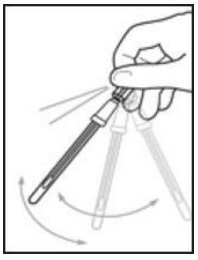
2. Aseptically transfer enriched sample from Enrichment Device to Detection Device.

3



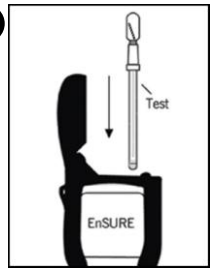
3. Activate Detection Device by breaking Snap-Valve. Squeeze bulb to release liquid into tube. Liquid should now be in bottom of tube.

4



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

5



5. Insert Detection Device into a luminometer and initiate measurement.

6

6. Record results as RLUs and refer to Table 2 to interpret results.

Instructional Video:
www.youtube.com/HygienaTV