MicroSnap® Total

For using:

Enrichment options

- Product No. MS1-TOTAL (MicroSnap® Total Enrichment Device)
- Product No. MS1-N-BROTH-9ML (MicroSnap® Enhanced Nutrient Broth in 9 mL Vial)

Detection

- Product No. MS2-TOTAL (MicroSnap® Total Detection Device)

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 031501

Introduction

Description and Intended Use

MicroSnap® Total is a rapid bioluminogenic test for the detection and enumeration of the total population of viable aerobic bacteria in a sample and provides 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 Hygiena® luminometer. For challenging matrices such as opaque liquid suspensions, we offer 9 mL vials containing a proprietary Enhanced Nutrient Broth for use instead of the incubation device.

The two-step test procedure requires a short incubation period facilitating the growth of bacteria followed by a detection step. During the incubation, bacterial numbers increase, and sample interference is reduced. As bacteria grow, they use up the 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 the 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 the initial starting concentration of bacterial contamination in the pre-enriched sample.

Intended User

Laboratory personnel trained in standard microbiological practices are qualified to use MicroSnap Total.

Applicability

MicroSnap Total is applicable for the enumeration of metabolically viable aerobic bacteria from environmental surfaces, product samples and liquids. The method (MicroSnap Enrichment Device with MicroSnap Detection Device) was validated through the AOAC Research Institute *Performance Tested Methods*℠ (*PTM*) Program for a wide range of foods, including major food groups such as meat, dairy and vegetables. For details, refer to AOAC RI *PTM* Certificate 031501 at www.hygiena.com/documents.

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 to 25 °C) prior to use with MicroSnap devices. Samples that are not brought to ambient temperature before incubation (e.g., taken directly from refrigeration ~4 °C) will under-detect due to the time lag in reaching the incubation temperature.

It is important that all media or diluents used with MicroSnap Total are sterile. Inhibitors in media and diluents are the prime reason for most unsuccessful detections. Hygiena recommends the diluents listed in the Required Materials section.

Additional Required Materials from Hygiena (Not Provided)

- EnSURE® Touch or SystemSURE *Plus*® luminometer (Product No. ETOUCH or SS3, respectively)
- Dry Block Incubator (at 30 ± 0.5 °C or 32 ± 0.5 °C) (Product No. INCUBATOR or INCUBATOR2)
- Block options for incubators:
  - 35-wells for swabs for INCUBATOR2 (Product No. IB001)
  - 15-wells for 9 mL vials for INCUBATOR2 (Product No. IB002)
  - 12-wells for swabs for INCUBATOR (Product No. IB003)
  - 6-wells for 9 mL vials for INCUBATOR (Product No. IB004)

Required Materials When Testing Product Samples (Not Provided)

- Sample bags
- Homogenizing equipment
- Pipettor and tips for 1 mL and 0.1 mL
- Product sample diluent options:
  - Buffered peptone water
  - Maximum recovery diluent (Note: Maximum recovery diluent was used for the AOAC RI *PTM* validation study)
  - Butterfield's diluent
  - Sterile water
- Optional when using Enhanced Nutrient Broth: vortex

Important Tips Before Starting the Test

- For challenging samples (e.g., opaque solutions; samples that may contain sanitizers, surfactants or other inhibitory compounds), use the MicroSnap Nutrient Broth for enrichment (for details, see Appendix and diagrams).
- Product samples can be stored prior to use at 4 °C for up to 2 days but must be equilibrated to room temperature (20 to 25 °C) before testing samples with MicroSnap Total.
- MicroSnap Total Enrichment Devices, MicroSnap Nutrient Broth Vials and MicroSnap Total Detection Devices must be equilibrated to room temperature before use.
- Use aseptic techniques: when collecting samples or transferring enriched samples, do not touch the swab or the inside of the Enrichment Device or Vial with your fingers.

Test Procedure

Step 1: Incubation with the MicroSnap Total Enrichment Device

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

1. Collect and prepare the sample, according to sample type as noted:
    a. Surface Samples—Use the pre-moistened Enrichment Device to sample a 4 x 4 inch (10 x 10 cm) square area.

        Important swabbing technique tips:

        i. For irregular surfaces, ensure the swabbing technique remains consistent for each test and swab a large enough area to collect a representative sample.

        ii. Swab in a crisscross pattern vertically, horizontally and diagonally in both directions.

        iii. Rotate the swab while collecting the sample to maximize sample collection on the swab tip.

        iv. Apply sufficient pressure to create flex in the swab shaft.

    b. Liquid Samples—Transfer 1 mL of a liquid or water sample directly to the Enrichment Device.

    c. Solid Product Samples—Transfer 1 mL of an appropriate suspension, e.g., 10% w/v food homogenate, directly to the Enrichment Device.

        i. Food homogenate should be prepared by weighing 10 or 50 g of food matrix and adding it to a stomacher bag containing 90 mL or 450 mL of diluent, respectively.

        ii. For unknown sample contamination, prepare and test 1:10 serial dilutions (i.e., 10%, 1% and 0.1%).

        iii. If replicate samples are required, then another 10 g or 50 g should be removed from the bulk matrix and the dilution series repeated. Replication can be achieved by drawing multiple 1 mL aliquots from either the 10%, 1% or 0.1% dilutions depending on relative light units (RLUs) achieved.

            Note: When performing comparison testing, sample assays must be started within 10 minutes of each other for comparable results between methods.

2. Re-attach the swab back into the swab tube. The device should look the same as it did when first removed from the bag.
3. Activate the Enrichment Device by holding the swab tube firmly and using your thumb and forefinger to break the Snap-Valve by bending the bulb forward and backward.
4. Separate the bulb and swab tube until the swab tip is above the fluid and squeeze the bulb to flush all the media into the swab tube. Ensure most of the broth is at the bottom of the swab tube.
5. Re-attach the swab back into the swab tube firmly to seal the device and shake the tube gently to mix the sample and broth.
6. Incubate at 30 ± 0.5 °C for 7 hours ± 10 minutes.

Step 2: Detection

The detection procedure is described below and is also shown in diagrams (MicroSnap Enrichment Device or MicroSnap Enhanced Nutrient Broth Vial).

Before beginning Step 2, turn on the luminometer. If you have programmed your MicroSnap sample in the luminometer, open the test screen of the sample you want to test.

Remember to equilibrate the MicroSnap Total Detection Device to room temperature (10 minutes at 20 to 25°C) before use.

1. Shake the test device by either tapping on the palm of your hand 5 times or forcefully flicking in a downward motion once.

   This is necessary to bring the liquid to the bottom of the tube, which will facilitate mixing of the enriched sample with the extractant in the tube.

2. Aseptically transfer 0.1 mL (2 drops) of enriched sample to the Detection Device:

   a. For MicroSnap Enrichment Devices, use the built-in dropper tip as a pipette:

      i. Squeeze and release the Enrichment Device bulb to mix and draw the sample into the bulb.

      ii. Aseptically open the Enrichment Device and the Detection Device by twisting and pulling to remove the bulbs.

      iii. Insert the Enrichment Device swab tip 1 inch (3 cm) into the top of the Detection Device tube and lightly squeeze the Enrichment Device bulb to transfer 2 drops of the enriched sample into the tube.

      Note: A fill line is added to the tube as a reference. Inconsistent transfer volumes increase the variation of the test results.

   b. For MicroSnap broth vials:

      i. Remove the Enhanced Nutrient Broth Vial from the incubator then shake or vortex for 10 seconds to disperse the sample.

      ii. Aseptically uncap the vial and open the Detection Device by twisting and pulling to remove the bulbs.

      iii. Aseptically pipette 0.1 mL of the enriched sample directly into the Detection Device tube.

   c. Reassemble the Enrichment Device to its original state or recap the Vial and return the sample to the incubator for potential retesting.

      Note: When testing replicates from the same enriched sample, all replicates must be performed within 10 minutes of each other to obtain comparable results.

3. Activate the Detection Device by holding the tube firmly and using your thumb and forefinger to break the Snap-Valve by bending the bulb forward and backward. Squeeze the bulb 3 times to release all the liquid to the bottom of the tube.

4. Shake gently for 2 seconds to mix.

5. Immediately insert the whole device into the luminometer, close the lid and while holding the unit upright, press the button to initiate the measurement.

6. Results will appear after 10 or 15 seconds, depending on the instrument you are using:

   a. EnSURE Touch luminometers display results in CFUs in 10 seconds. MicroSnap samples can be programmed directly on the luminometer or by using the SureTrend® software.

   b. SystemSURE *Plus* luminometers display results in RLUs in 15 seconds. Use SureTrend® 4 to program MicroSnap samples and set RLU thresholds on the luminometer to correspond with the required colony forming unit (CFU) limits.

Additional Information

Potential Limit of Detection

The 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 the incubation time increases. At 7 hours, the detection level approaches 10 to 100 CFU per mL of incubation media, and the dynamic range of MicroSnap Total in the EnSURE Touch luminometer is proportional to the actual range of RLU feasible in the EnSURE Touch instrument (Table 1).

Table 1. Potential Dynamic Range at a 7-hour Incubation with the EnSURE Touch Luminometer.

Sample Type	CFU Range*
Surface (4 x 4 inches)	10 – 1,000,000 CFU/swab
Liquid (1 mL)	10 – 600,000 CFU/mL
Suspension of solid (10% w/v)	100 – 60,000 CFU/g†

* Additional factors, such as dilutions, incubation times and matrix types can alter the ranges shown in Table 1.

† For samples where contamination is above the ranges detailed in Table 1, the following serial dilutions must be made before reading results on the luminometer:

- 1% suspension will give a range of 1,000 – 600,000 CFU
- 0.1% suspension will give a range of 10,000 – 6,000,000 CFU

Note: When testing multiple serial dilutions, all dilutions must be prepared and tested within 10 minutes of each other to obtain linear results.

AOAC RI *Performance Tested Methods*℠ Certification

The detection of aerobic heterotrophic bacteria using the MicroSnap Total System (i.e., MicroSnap Enrichment Device and the MicroSnap Detection Device) with the EnSURE Touch luminometer has earned AOAC RI *PTM* Certification (License #031501) from the AOAC Research Institute.

Food matrices (Table 2) 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 Total at 7-hour incubations.

Table 2. Validated Matrices with MicroSnap Enrichment* and Detection Devices.

Quantitative (50 g with 7 hour ± 10 Minute Enrichment Time)
Fresh ground beef (<20% fat)
Raw chicken
Raw cow's milk
Fresh cream cake (17% fat)
Pre-packaged iceberg leaves

* MicroSnap Enrichment Device (1 mL enrichment volume)

Interpretation of Results

Results on EnSURE Touch luminometers are shown in CFUs, providing quantitative results as CFU/g or CFU/mL.

Results on SystemSURE *Plus* luminometers are displayed as RLUs. The numerical RLU output is proportional to the 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 CFUs. SystemSURE *Plus* luminometers have a 4-digit RLU output display, and results ≥10,000 RLU will be outside the display range.

Where several dilutions are prepared and tested for samples with unknown contamination, the CFU/g or CFU/mL is calculated by multiplying the CFU result by the corresponding dilution factor. A convenient RLU-to-CFU conversion tool that can account for dilution factors is available for SystemSURE *Plus* luminometers (contact Hygiena's technical services team). EnSURE Touch makes this correlation easier because its software does the conversion for you, using data generated from the AOAC Validation Studies as well as additional internal testing.

Troubleshooting

Table 3 provides guidance on how to overcome some commonly seen sample effects. For additional protocol or matrix support, contact us at www.hygiena.com/support.

Table 3. Troubleshooting

Observation	Possible Cause	Recommended Action
Uncharacteristically high CFUs with some matrices, such as leafy greens	Some sample types naturally contain high levels of nucleotides that can increase CFU results.	Contact us for assistance with customizing the RLU-to-CFU conversion and the instrument threshold levels for your sample matrix.
Uncharacteristically low CFUs with thick, opaque or dark sample matrices, such as undiluted milk or chocolate	Interference with light detection by the luminometer can be caused by a blanching effect from the sample matrix.	Use the MicroSnap Enhanced Nutrient Broth in 9 mL vials for enrichment. See Appendix for details.

Calibration and Controls

It is advisable to run positive and negative controls according to Good Laboratory Practice. Hygiena offers the following calibration verification device: CalCheck LED Calibration Verification Device (Product No. CAL).

Storage and Shelf Life

- Store at 2 to 8 °C (36 to 46 °F).
- Do not use past the expiration date on the label.

Disposal

Disinfect before disposal. MicroSnap devices can be disinfected by autoclaving or by soaking unsealed devices 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 and Precautions

- MicroSnap device components do not pose any health risks when used correctly. Used devices confirming positive results may be a biohazard and should be disposed of safely in compliance with Good Laboratory Practices and Health and Safety Regulations (see disposal instructions above).
- Devices and vials are designed for single use. Do not reuse.
- Sampling should be done aseptically to avoid cross-contamination.

- Avoid prolonged exposure to light.
- Verify proper incubation temperature and time for the test application.
- The incubation time for quantitative (enumeration) results is 7 hours ± 10 minutes as specified in the above instructions unless you have been directed otherwise by Hygiena's R&D team for custom applications that require different incubation times (or temperatures).
- Ensure proper sample dilution so that samples can be read within the luminometer's dynamic range.
- When testing multiple serial dilutions, all dilutions must be prepared and tested within 10 minutes of each other to obtain linear results.
- When testing replicates from the same enriched sample, all replicates must be performed within 10 minutes of each other to obtain comparable results.
- When performing comparison testing, sample assays must be started within 10 minutes of each other for comparable results between methods.

Caution and User Responsibility

- MicroSnap devices have not been tested with all possible food products, food processes, testing protocols or with all possible microorganism strains.
- Do not use this test for diagnosis of conditions in humans and animals.
- 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.
- It is the user's responsibility when selecting a test method to evaluate a sufficient number of samples.
- As with any culture medium-based test, MicroSnap results do not constitute a guarantee of product quality.
- Personnel must be trained in proper testing techniques and standard microbiological practices.

Hygiena Liability

As with any culture medium-based test, 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 the product to Hygiena; contact Customer Service for a Returned Goods Authorization Number.

Contact Information

For more information, visit www.hygiena.com/contact. For technical support, visit www.hygiena.com/support.

Appendix:
Enrichment of Challenging Matrices with MicroSnap Enhanced Nutrient Broth

MicroSnap Enhanced Nutrient Broth contains 9 mL of a unique liquid medium designed to grow aerobic and facultative microorganisms while enhancing the production of biomarkers and specific enzymes diagnostic of coliforms and *E. coli* and reducing sample interferences. The broth is primarily intended for applications requiring the detection of bacteria in opaque liquid suspensions (e.g., coliforms in pasteurized milk).

MicroSnap Enhanced Nutrient Broth is a ready-to-use media compatible with MicroSnap Total (MS2-TOTAL), MicroSnap Coliform (MS2-COLIFORM) and MicroSnap *E. coli* (MS2-ECOLI) Detection Devices. Instructions in this insert are for enriching milk, opaque solutions and other challenging food samples. For help developing a protocol for your matrix, including adjusting enrichment incubation temperatures, contact Hygiena for guidance.

Important Tips Before Starting the Test

- Visually inspect the liquid in the vial before use. The liquid should be clear and light straw color, not turbid or cloudy.
- Use a permanent marker to identify the sample on the vial label.

Step 1: Enrichment with MicroSnap Enhanced Nutrient Broth

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

1. Collect and prepare the sample using aseptic techniques:
    a. Liquid Samples—Add 1 mL of sample directly to the vial of Enhanced Nutrient Broth.
    b. Solid Samples—Transfer 1 mL of a suitable sample dilution in sterile diluent directly to the vial of Enhanced Nutrient Broth.
2. Replace and tighten the cap.
3. Shake or vortex for 10 seconds to mix contents.
4. Incubate the vial in a Hygiena Digital Dry Block Incubator for 7 hours ± 10 minutes at the appropriate temperature for your sample type (Table 4).

Table 4. Temperature and Potential Dynamic Range for a 7-hour Incubation.

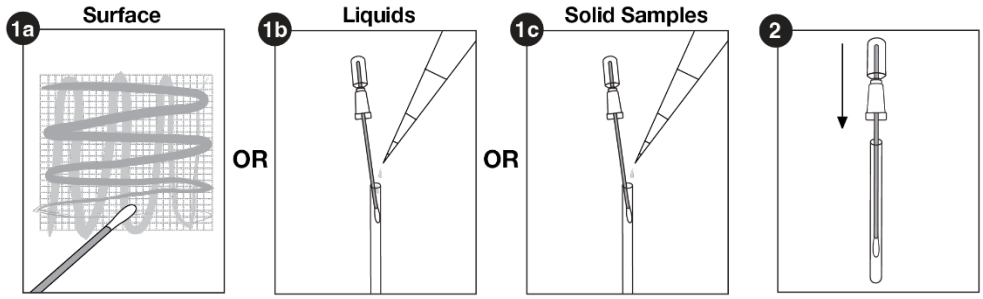
Sample Type	Incubation Temperature (°C)	CFU Range
Milk	32 ± 0.5	50 – 25,000
Liquid or solid food product	30 ± 0.5	

Step 2: Detection

Follow instructions for detection as described above.

MicroSnap® Total Enrichment and Detection Devices

Step 1: Sample Enrichment

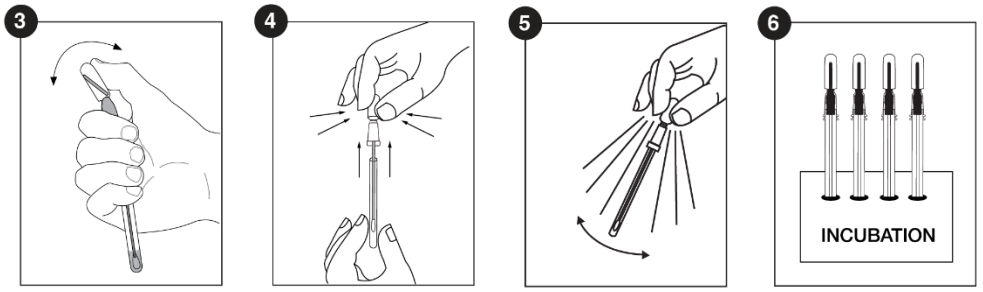


1a Surface: Swab a 10 x 10 cm area with room-temperature (RT) Enrichment Device.

1b Liquids: Add 1 mL of liquid food, beverage or water directly to RT Enrichment Device.

1c Solid Samples: Add 1 mL of appropriate dilution of solid sample directly to RT Enrichment Device.

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



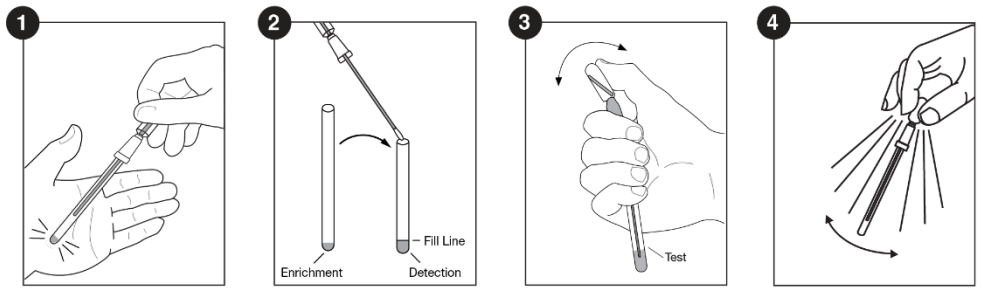
3. Activate Device. Bend bulb, breaking Snap-Valve.

4. Lift bulb up (1 – 2 inches) and squeeze to release liquid into bottom of tube.

5. Replace bulb into tube and shake tube gently to mix sample in liquid.

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

Step 2: Detection or Measurement

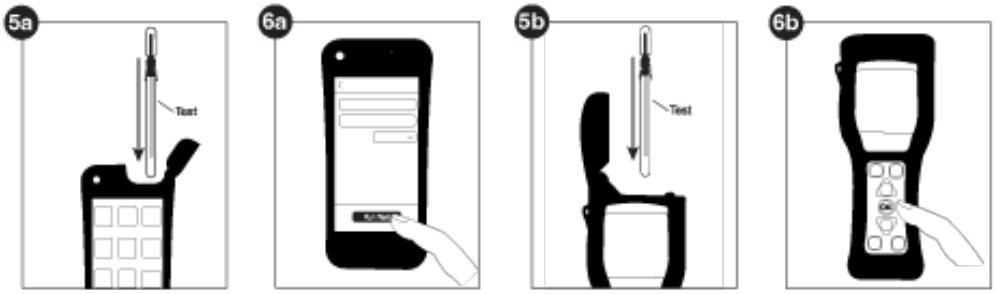


1. Equilibrate Detection Device to room temperature. Shake to bring liquid to bottom.

2. Aseptically transfer 2 drops (0.1 mL) of sample from Enrichment Device to Detection Device.

3. Activate Detection Device (Test) by breaking Snap-Valve. Squeeze bulb to release liquid into tube.

4. Shake tube gently to mix sample in liquid.



5a. **EnSURE® Touch**: In MicroSnap® application: If sample is programed, select sample; otherwise, select **Quick Test**. Then, press **Run Test**.

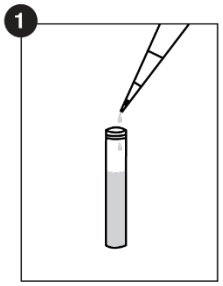
6a. **EnSURE Touch:** Automatically saves results. Register and sync luminometer wirelessly to the SureTrend® software to see reports and datasets.

5b. **SystemSURE *Plus*®:** Insert Detection Device and press **OK** to initiate measurement.

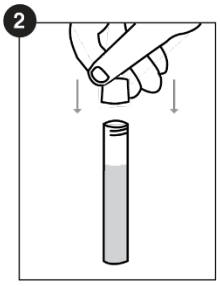
6b. **SystemSURE *Plus*:** Record RLU results and convert to CFUs.

MicroSnap® Enhanced Nutrient Broth Vial and MicroSnap Detection Device

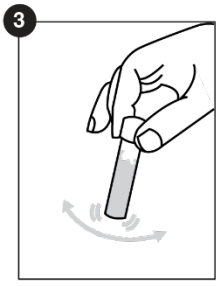
Step 1: Sample Enrichment



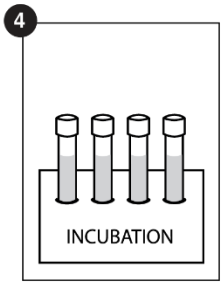
1 Equilibrate sample and broth at 20 to 25 °C. Add 1 mL of appropriate dilution of samples to Enhanced Nutrient Broth.



2. Replace and tighten cap.

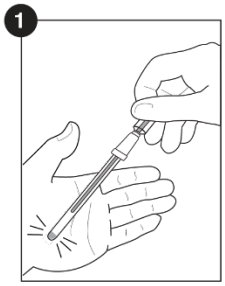


3. Shake or vortex for 10 seconds.

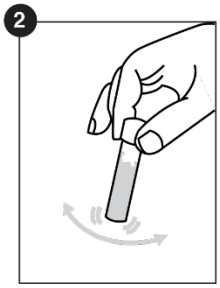


4. Incubate at 30 ± 0.5 °C (food product) or 32 ± 0.5 °C (milk) for 7 hours ± 10 minutes.

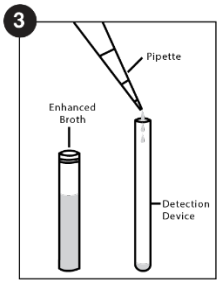
Step 2: Detection or Measurement



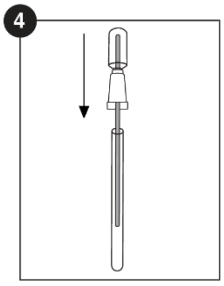
1. Equilibrate Detection Device to room temperature. Shake to bring liquid to bottom.



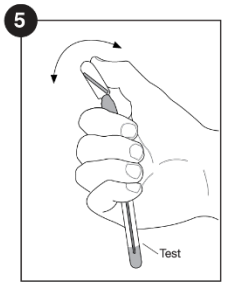
2. Shake or vortex for 10 seconds.



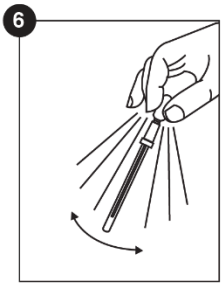
3. Aseptically transfer 0.1 mL of enriched sample to the Detection Device.



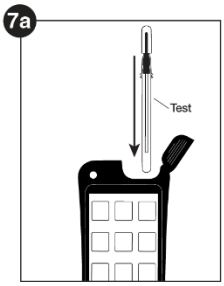
4. Reassemble Detection Device to original state.



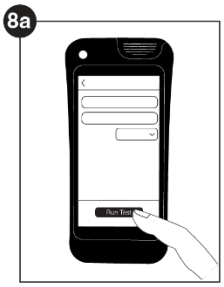
5. Activate device by breaking Snap-Valve. Squeeze bulb to release liquid into tube.



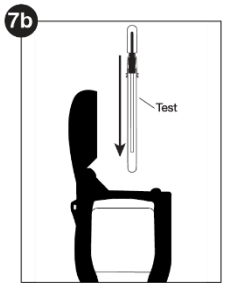
6. Shake tube gently to mix sample in liquid.



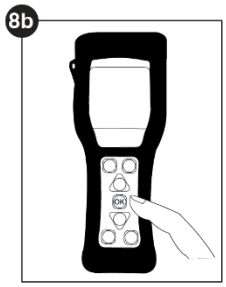
7a. **EnSURE® Touch**: Insert device into EnSURE Touch. In MicroSnap® application: If sample is programed, select sample; otherwise, select **Quick Test**. Then, press **Run Test**.



8a. **EnSURE Touch**: Automatically saves results. Register and sync luminometer wirelessly to the SureTrend® software to see reports and datasets.



7b. **SystemSURE *Plus*®**: Insert Detection Device and press **OK** to initiate measurement.



8b. **SystemSURE *Plus***: Record RLU results and convert to CFUs.