MicroSnap® Coliform and *E. coli*

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 071302

For using:

Enrichment options

- Product No. MS1-CEC (MicroSnap® Coliform & *E. coli* Enrichment Device)
- Product No. MS1-N-BROTH-9ML (MicroSnap® Enhanced Nutrient Broth in 9 mL Vials)
- Product No. MS1-EB-BROTH-9ML (MicroSnap® Enhanced EB Broth in 9 mL Vials)

Detection options

- Product No. MS2-COLIFORM (MicroSnap® Coliform Detection Device)
- Product No. MS2-ECOLI (MicroSnap® *E. coli* Detection Device)

Introduction

Description and Intended Use

MicroSnap® Coliform and *E. coli* are rapid bioluminogenic tests for the detection and enumeration of coliforms and *Escherichia coli* in products and environmental samples in 6 or 8 hours. MicroSnap Coliform and *E. coli* consist of an Enrichment Device containing a specific growth medium and a Detection Device containing a bioluminogenic substrate in which the detection reaction is measured using a hand-held luminometer from Hygiena®.

The two-step test procedure requires a short incubation period facilitating recovery of bacteria followed by a detection step. The incubation time for a sample is determined by the level of sensitivity required. During incubation, the bacteria use available food resources in the media and produce more beta-galactosidase and beta-glucuronidase, which are the diagnostic enzymes required for the bioluminogenic reaction.

After incubation, a small amount of enriched solution from the Enrichment Device is transferred to the Detection Device, activated, incubated for 10 minutes and then measured in an EnSURE® Touch luminometer. Specific substrates react with diagnostic enzymes to produce light. The greater the number of bacteria in the sample, the higher the biomarker concentration and the greater the output of light. Light is measured in a few seconds. The unit of measurement is colony forming units (CFUs). Light output is directly proportional to the initial starting concentration of bacterial contamination in pre-enriched samples.

Some matrices, such as opaque liquid suspensions or samples with extreme pH values, may require dilution. In these cases, we offer 9 mL vials containing proprietary broths for use in place of the Step-1 enrichment device. Use of Enhanced Broths in 9 mL vials is not included under the AOAC Research Institute (RI) *Performance Tested Methods*℠ (*PTM*℠) certification.

The Enhanced EB Broth (9 mL vial) is more selective for coliforms and *E. coli* in the presence of lactic acid bacteria (LAB) or *Enterobacteriaceae*, while the Enhanced Nutrient Broth (9 mL vial) provides better recovery of aerobic bacteria, coliforms and *E. coli*. Guidance for selecting a MicroSnap broth is summarized in Table 1.

Table 1. MicroSnap Enhanced Broth Selection Guide.

Testing Needs	Vial Type
Total & Coliform/*E. coli*	Enhanced Nutrient Broth
Enterobacteriaceae & Coliform/*E. coli*	Enhanced EB Broth
Coliform/*E. coli* only – low background flora	Enhanced Nutrient Broth
Coliform/*E. coli* – high background flora	Enhanced EB Broth

Intended User

Laboratory personnel trained in standard microbiological practices are qualified to use MicroSnap Coliform and *E. coli* devices.

Applicability

MicroSnap Coliform and *E. coli* is applicable for the enumeration of coliform and *E. coli* from environmental surfaces, product samples, water and other filterable liquids. The method was validated through AOAC RI *PTM* certification for a variety of foods, including meat, dairy, seafood, vegetables and beverage food groups. For details, refer to AOAC RI *PTM* Certificate 071302 at www.hygiena.com/documents.

Required Materials (Not Provided)

- EnSURE Touch luminometer (Product No. ETOUCH)
- Dry Block Incubator (at 37 ± 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
- 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

Important Tips Before Starting the Test

- For samples that may require dilution (e.g., opaque solutions; samples that may contain sanitizers, surfactants or other inhibitory compounds), use the MicroSnap Enhanced EB Broth or Nutrient Broth for enrichment (for details, see Appendix and diagrams).
- Product samples can be stored prior to use at 2 to 8 °C for up to 2 days but must be equilibrated to room temperature (20 to 25 °C) before testing samples with MicroSnap Coliform and *E. coli*.
- Enrichment devices or vials (i.e., MicroSnap Coliform and *E. coli* Enrichment Devices, MicroSnap Enhanced EB Broth and MicroSnap Enhanced Nutrient Broth) and detection devices (i.e., MicroSnap Coliform Detection Devices and MicroSnap *E. coli* Detection Devices) must be equilibrated to 20 to 25 °C 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: Enrichment

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 10 x 10 cm (4 x 4 inch) square area.

      Important swabbing technique tips:

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

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

   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 should be repeated. Replication can be achieved by drawing multiple 1 mL aliquots from either the 10%, 1% or 0.1% dilutions, depending on the CFUs achieved.

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

   d. Re-attach the swab to the swab tube. The device should look the same as it did when first removed from the bag.

2. 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.

3. 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.

4. Re-attach the swab to the swab tube firmly to seal the device and shake the tube gently to mix the sample and broth.

5. Incubate at 37 ± 0.5 °C for 6 hours ± 10 minutes for quantitative results (enumeration) or 8 – 24 hours for qualitative results (absence/presence).

   Note: In the qualitative AOAC RI *PTM* validation studies, samples were incubated for 8 hours ± 10 minutes.

Step 2: Detection

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

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 Coliform or *E. coli* Detection Device (MS2-COLIFORM or MS2-ECOLI) 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.

   Note: This is necessary to bring the liquid to the bottom of the tube, which will facilitate the 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 3 cm (1 inch) into the top of the Detection Device tube and gently 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 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 bulb.

      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. Incubate the Detection Device for 10 ± 0.5 minutes at 37 ± 0.5 °C.

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

7. EnSURE Touch luminometers display results in CFUs in 10 seconds.

   Note: MicroSnap samples can be programmed directly on the luminometer or by using SureTrend® software.

Additional Information

Further Tests

If a positive result is found using the MicroSnap Coliform Detection Device (MS2-COLIFORM), then confirm the presence or absence of *E. coli* in the sample by repeating Step 2: Detection instructions above using another aliquot from the same enriched sample and the MicroSnap *E. coli* Detection Device (MS2-ECOLI). If performing *E. coli* tests only, an additional confirmatory test should be considered, such as PCR on the Hygiena BAX® System.

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.

Table 2. Potential Dynamic Range (Limit of Detection) for the EnSURE Touch Luminometer.

Sample Type	CFU Range* (Enrichment: 6 h ± 10 min)	CFU Presence or Absence (Enrichment: 8 ± 10 min)†
Surface	10 – 10,000 CFU/swab	0 (absence) 1 CFU (caution) ≥2 CFU (presence)
Liquid (1 mL)	10 – 10,000 CFU/mL	
Suspension of solid (10% w/v)	100 – 10,000 CFU/g	

* Additional factors, such as dilutions, incubation times and matrix types, can alter the ranges shown in this table. If sample contamination is above the ranges detailed in this table, then dilutions should be made so that the contamination is within the detectable range of the luminometer. For example:
- 1% suspension will be 1,000 – 100,000 CFU for a 6-hour incubation.
- 0.1% suspension will be 10,000 – 1,000,000 CFU for a 6-hour incubation.

† Incubation for presence/absence results can be extended up to 24 hours. **Note**: The enrichment time was 8 hours ± 10 minutes for the qualitative AOAC RI *PTM* validation studies.

Interpretation of Results

Results on EnSURE Touch luminometers are shown in CFUs, providing qualitative (presence/absence) as well as quantitative (CFU/g or CFU/mL) results.

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. The EnSURE Touch software does this conversion, using data generated from the AOAC RI *PTM* validation studies as well as additional internal testing.

AOAC RI *Performance Tested Methods* Certification

The detection of coliform and *E. coli* using the MicroSnap Coliform and *E. coli* System (i.e., MicroSnap Coliform and *E. coli* Enrichment Device with either the MicroSnap Coliform Detection Device or the MicroSnap *E. coli* Detection Device) and Hygiena luminometers has earned AOAC RI *PTM* Certification (License #071302) from the AOAC Research Institute.

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 071302

Foods tested under AOAC RI *PTM* validation are listed in Table 3.

Table 3. Validated Matrices with MicroSnap Coliform and *E. coli* on Hygiena Luminometers.

Quantitative (Enrichment: 6 hours ± 10 minutes)		Qualitative (Enrichment: 8 hours ± 10 minutes)	
Coliform	***E. coli***	**Coliform**	***E. coli***
Raw ground beef	Raw ground beef	Raw ground beef	Raw ground beef
BLT sandwich	BLT sandwich	—	BLT sandwich
Raw cod	Raw cod	Raw cod	Raw cod
Cooked chicken	Cooked chicken	Cooked chicken	Cooked chicken
Lettuce	Lettuce	Lettuce	—
Milk	Milk	Milk	Milk
Raw chicken	Raw chicken	Raw chicken	Raw chicken
RTE ham	RTE ham	—	—
Raw prawns	Raw prawns	Raw prawns	Raw prawns
Bottled water	Bottled water	Bottled water	Bottled water

Additional Hygiena Validations

In addition to the matrices included in AOAC RI *PTM* certification studies (Table 3), Hygiena laboratories continue to test other matrices, such as chocolate milk and pasteurized milk. Optimal testing for some matrices involved use of the Enhanced EB Broth or Enhanced Nutrient Broth vials (9 mL), which were not included under the AOAC RI *PTM* certification. For qualitative testing, some users have validated extended incubation times (up to 24 h), which is not included under AOAC RI *PTM* certification.

Contact technical support at www.hygiena.com/support for information and assistance with additional matrices.

Limitations

It is important that samples are brought to room temperature (20 to 25 °C) before use in MicroSnap devices. Samples that are not brought to ambient temperature before incubation (e.g., taken directly from refrigeration at 2 to 8 °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 Coliform and *E. coli* 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.

The enzymatic profiles of some coliforms, such as strains of *Hafnia alvei*, are different from most other coliforms, so these strains will not be detected and enumerated by biochemical and chromogenic detection methods such as those used in MicroSnap Coliform testing.

Organisms with similar enzymatic profiles, such as *Shigella sonnei,* cannot be differentiated from *E. coli* by biochemical or chromogenic detection methods such as those used in MicroSnap *E. coli* testing.

In addition, the enzymatic profiles for some Shiga toxin-producing *E. coli* (STEC), such as *E. coli* O157:H7, means that they will be detected and enumerated by MicroSnap Coliform testing, but not MicroSnap *E. coli* testing.

For additional details, see Caution and User Responsibility or contact us.

Troubleshooting

Table 4 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 4. Troubleshooting.

Observation	Possible Cause	Recommended Action
Uncharacteristically high CFUs with some matrices, such as leafy greens and fermented dairy products.	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 one of the MicroSnap Enhanced Broths 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 bleaching (soak 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 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).
- Avoid prolonged exposure to light.
- Devices are designed for a single use. Do not reuse.

Caution and User Responsibility

- MicroSnap devices have not been tested with all possible food products, food processes, testing protocols or with all possible strains of the Coliform family.
- For *in vitro* use only. Not for use in diagnostic procedures.
- As with any culture medium-based test, MicroSnap results do not constitute a guarantee of product quality.

- 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.
- Sampling should be done aseptically to avoid cross-contamination.
- It is the user's responsibility when selecting a test method to evaluate a sufficient number of samples.
- Verify proper incubation temperature and time for the test application.
- The incubation time will be 6 hours ± 10 minutes for quantitative results (enumeration) or 8 – 24 hours for qualitative results (presence/absence) 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).

  Note: In the qualitative AOAC RI *PTM* validation studies, samples were incubated for 8 hours ± 10 minutes.
- 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.

Hygiena Liability

As with any culture medium-based test, MicroSnap Coliform and *E. coli* 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 the 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.

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Appendix: Enrichment of Challenging Matrices with MicroSnap Enhanced EB Broth or MicroSnap Enhanced Nutrient Broth

MicroSnap Enhanced Broths 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 broths are intended for applications requiring the detection of bacteria in challenging food samples, such as opaque liquid suspensions (see Table 1).

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. MicroSnap Enhanced EB Broth is a ready-to-use media compatible with MicroSnap EB (MS2-EB), 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 coliform and *E. coli* testing. For help developing a protocol for your matrix, including adjusting enrichment incubation temperatures, contact Hygiena for guidance.

Important Tips Before Starting the Test

- The use of the Enhanced Broths in 9 mL vials is not included under AOAC RI *PTM* certification.
- Visually inspect the liquid in the vial before use. Liquid should be clear and a 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 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 Broth.
   b. Solid Samples—Transfer 1 mL of a suitable sample dilution in sterile diluent directly to the vial of Enhanced 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 6 or 8 hours, depending on your sample type and the sensitivity required (Table 5).

Table 5. Incubation Time, Temperature and Potential Dynamic Range.

Incubation Time*	CFU Range	Enhanced Nutrient Broth		Enhanced EB Broth
		Milk	Liquid or Solid Food	Milk, Liquid or Solid Food
		32 ± 0.5 °C	30 ± 0.5 °C	37 ± 0.5 °C
6 h ± 10 min	50 – Log 6	Enumeration	Enumeration	Enumeration
8 h ± 10 min†	<5 – 5,000	Presence/absence	Presence/absence	Presence/absence

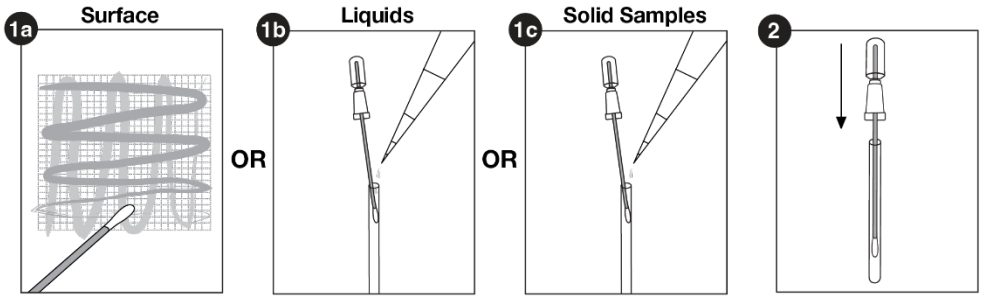
* Enumeration for Incubation periods outside of defined times have not been validated.
† Incubation for presence/absence results can be extended up to 24 hours.

Step 2: Detection

Follow instructions for detection as described above.

MicroSnap® Enrichment and Detection Devices for Coliform and *E. coli*

Step 1: Sample Enrichment

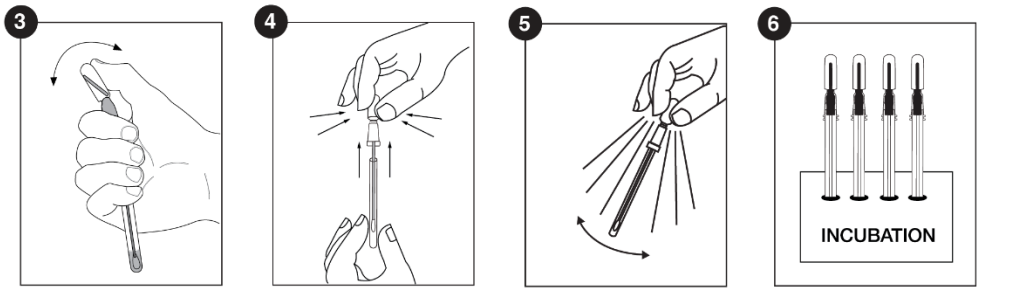


1a. Surface: Swab a 10 x 10 cm area with room-temperature* (RT) Enrichment Device.

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

1c. Solid Samples: Add 1 mL of 10% w/v suspension of solid sample directly to RT Enrichment Device.

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



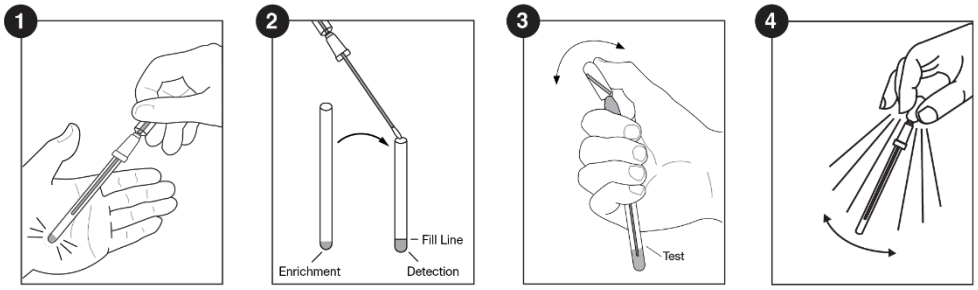
3. Activate Device. Bend bulb, breaking Snap-Valve.

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 37 ± 0.5 °C for 6 h ± 10 min (quantitative) or 8 h ± 10 min (qualitative).

Step 2: Detection or Measurement

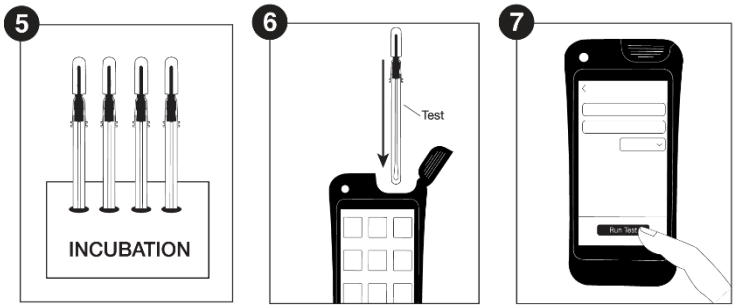


1. Equilibrate Detection device to room temperature. Shake to bring liquid to bottom.

2. Aseptically transfer 2 drops (0.1 mL) enriched 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.



5. Incubate Detection Device for 10 ± 0.2 minutes at 37 ± 0.5 °C.

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

7. EnSURE Touch automatically saves results.† Register and sync luminometer wirelessly to SureTrend® software to see reports and datasets.

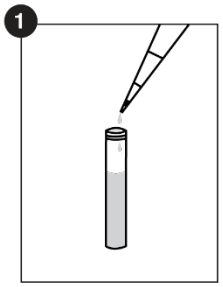
* Room temperature = 20 to 25 °C.

† If positive for Coliform, presence of *E. coli* can be verified by repeating Step 2 using another aliquot from the same enriched sample and an *E. coli* Detection Device.

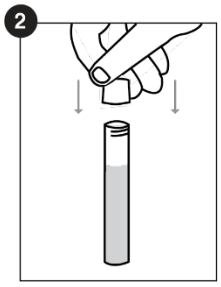
MicroSnap® Enhanced Broth Vial and MicroSnap Detection Device

Note: Use of the Enhanced Nutrient Broth is not included under AOAC RI *PTM* certification.

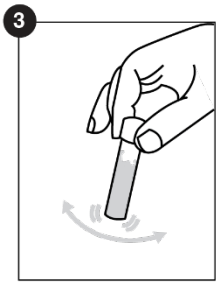
Step 1: Sample Enrichment



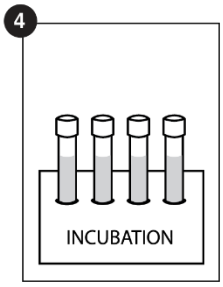
1. Equilibrate sample and broth to 20 – 25 °C. Add 1 mL of appropriate dilution of samples to Enhanced Broth.



2. Replace and tighten cap.

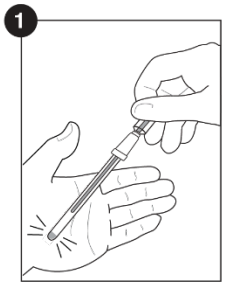


3. Shake or vortex for 10 seconds.

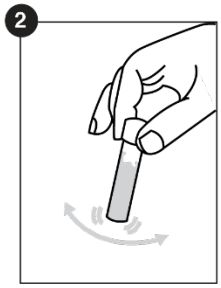


4. Incubate at the appropriate time and temperature for your sample type. Refer to Table 4 for details.

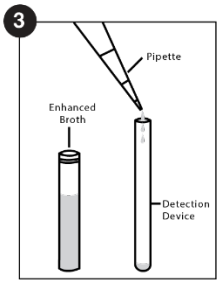
Step 2: Detection or Measurement



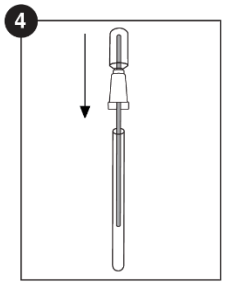
1. Equilibrate Detection Device to room temperature. Shake to bring liquid to bottom.



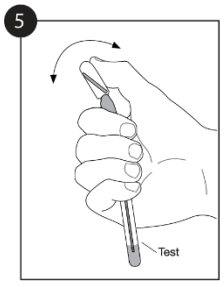
2. Shake or vortex for 10 seconds.



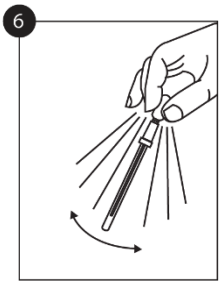
3. Aseptically transfer 0.1 mL of enriched sample to the Detection Device.



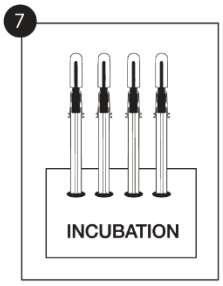
4. Reassemble Detection Device to original state.



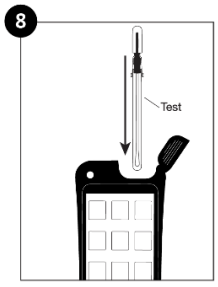
5. Activate device by breaking Snap-Valve. Squeeze bulb to release liquid into tube.



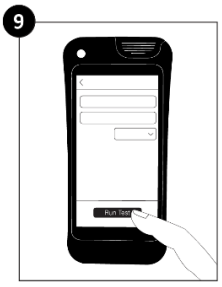
6. Shake tube gently to mix sample in liquid.



7. Incubate Detection Device for 10 ± 0.2 minutes at 37 ± 0.5 °C.



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



9. EnSURE Touch automatically saves results. Register and sync luminometer wirelessly to the SureTrend® software to see reports and datasets.