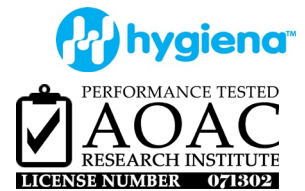


MicroSnap™ – Coliform and *E. coli*

Part Numbers:

- MicroSnap™ Coliform Incubation Device (Part # MS1-CEC)
- MicroSnap™ Coliform Detection Device (Part # MS2-COLIFORM)
- MicroSnap™ *E. coli* Detection Device (Part # MS2-ECOLI)



Description / Intended Use:

MicroSnap™ Coliform and *E. coli* is a rapid bioluminogenic test for detection and enumeration of Coliform and *E. coli* in products and environmental samples in 6 or 8 hours. MicroSnap™ Coliform and *E. coli* consists of an Incubation Device containing a specific growth medium and a Detection Device containing a bioluminogenic substrate in which the detection reaction is measured using a small portable luminometer.

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

After incubation, a small amount of enriched solution from the Incubation Device is transferred to Detection Device, activated, incubated for 10 minutes, and then measured in EnSURE™ Touch or EnSURE™. A specific substrate reacts with diagnostic enzymes to produce light. The greater the number of bacteria in the sample, the higher the biomarker concentration, and the greater output of light. Light is measured in seconds. The unit of measurement is in Relative Light Units (RLU) and/or a Colony Forming Units (CFU) depending on which Hygiena™ luminometer is used. Light output is directly proportional to initial starting concentration of bacterial contamination in pre-enriched samples.

MicroSnap™ can be used to test environmental surfaces, product samples, water, and other filterable liquids. MicroSnap™ has been validated for a wide range of foods including major food groups such as meat, dairy, vegetables, and beverages.

Intended User:

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

Applicability:

MicroSnap™ Coliform and *E. coli* is applicable for enumeration of Coliform and *E. coli* from environmental surfaces, product samples, water, and other filterable 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, vegetables, and beverages. Refer to AOAC-RI PTM Certificate #071302 for details.

Limitations:

It is important that samples are brought to ambient temperature (20-25°C) prior to use in 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™ Coliform and *E. coli* are sterile. Inhibitors in media and diluents are the prime reason for most unsuccessful detections. Hygiena™ recommends the diluents listed below.

Required Materials (Not Provided):

- EnSURE™ Touch or EnSURE™ luminometer
- Hygiena™ Dry Block Incubator at 37°C ± 0.5°C

Required Materials for 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:

Instructional Videos:

www.youtube.com/HygienaTV

www.vimeo.com/HygienaTV

Step 1: Incubation

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

Environmental surfaces and product samples:

1. Collect sample and transfer aliquot into MicroSnap™ Coliform & *E. coli* Incubation Device (MS1-CEC).
 - a. Samples Preparations Include:
 - i. Surface 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.
 1. Important swabbing technique tips:
 - a. Rotate swab while collecting sample to maximize sample collection on swab tip.
 - b. Apply sufficient pressure to create flex in swab shaft.
 - c. Swab in a crisscross pattern vertically, horizontally, and in both diagonal directions.
 - ii. Liquid – Transfer 1mL of liquid or water samples added directly to Incubation Device.
 - iii. Product - Transfer 1mL of 10% (1:10) w/v food homogenate into Incubation Device. Food homogenate should be prepared using industry recommended diluents and standard microbiological procedures (e.g., 50g in 450ml of diluent as used in AOAC validation studies). Other sample sizes should be validated by user.
 2. Re-attach the swab back into swab tube. Device should look the same as it did when first pulled from the bag.

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3. Activate Incubation 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 the broth is in bottom of swab tube.
5. Re-attach swab back into swab tube to firmly seal device. Shake tube gently to mix sample and broth.
6. Incubate at 37° ± 0.5°C.
7. For enumeration, incubate for 6 hours with enumerable range of 10 – 10,000 CFU/g (mL or 4x4 area).
8. For presence/absence, incubate for 8 hours.

Step 2: Detection:

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

1. Allow MicroSnap™ Coliform or *E. coli* 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 sample from Incubation Device to Detection Device using the sab in the Incubation Device as a pipette.
3. Aseptically transfer 0.1 mL of enriched solution by utilizing the built-in dropper tip of the Incubation Device.
 - a. Squeeze and release Incubation Device bulb to mix and draw sample into bulb.
 - b. 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.
 - c. 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 incubated sample, all replicates must be performed within 10 minutes to obtain comparable results.*
4. 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.
5. Shake gently for 2 seconds to mix.
6. Incubate Detection Device for 10 minutes (± 0.2 min) at 37°± 0.5°C.
7. 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.
 - a. EnSURE™ Touch gives results in 10 seconds. Results are shown in CFU (Colony Forming Units). MicroSnap™ samples can be programed directly on the luminometer or by using SureTrend Cloud.
 - b. EnSURE™ gives results in 15 seconds. Results are shown in RLU (Relative Light Units). Use SureTrend to program MicroSnap™ samples and set RLU thresholds on the EnSURE™ to correspond with required CFU limits. Refer to "Interpretation of Results" below for correlation.

Further Tests:

If a positive result is found using MicroSnap™ Coliform, then to confirm presence or absence of *E. coli* in sample, repeat "Step 2: Detection" instructions above using MS2-ECOLI Detection Device. If performing *E. coli* tests only, an additional confirmatory test should be considered such as PCR on the Hygiena™ BAX® System.

Interpretation of Results:

Results are displayed as Relative Light Units (RLU) on EnSURE™ and/or a +/- range of Colony Forming Units (CFU) on EnSURE™ Touch. Table 1 shows equivalent CFU values to RLU. This will tell you how many Coliforms or *E. coli* CFU were present in original sample.

Hygiena's luminometers approved for this test have different performance characteristics and sensitivities so interpretation will differ accordingly. EnSURE™ luminometers have a 4-digit RLU output display and results ≥10,000 RLU will be outside display range (See Table 1). EnSURE™ Touch can give qualitative (pass/fail) as well as quantitative results as CFU/g or CFU/mL.

Quantitative/Enumeration Measurements:

Incubation Time: 6 hours

Numerical results are proportional to enzyme content extracted from starting inoculum at each incubation timepoint. Compare RLU output with corresponding instrument in Table 1; data is derived from AOAC Validation study 2013. Percentage agreement between traditional methods and MicroSnap™ is greater than 92%. EnSURE Touch makes this correlation easier because its software does conversion for you, using the data generated from the AOAC Validation Studies as well as additional internal testing.

Table 1: Potential Relationship between CFU and MicroSnap™ Coliform and *E. coli* RLU

Estimated CFU	RLU on EnSURE™
<10	< 2
<20	< 4
<50	< 7
<100	< 12
<200	< 20
<500	< 35
<1,000	< 60
<5,000	< 180
<10,000	< 300

Qualitative/ Presence/Absence Measurements:

Incubation Time: 8 hours

Qualitative (presence/absence) measurements are usually used to detect low levels of contamination such as <10 CFU/g food or <1 CFU/100mL water. After sample preparation, inoculum for incubation step would either contain no bacteria or ≥1 CFU.

If incubated for 8 hours at 37°C, inoculums of 1 CFU will create enough enzymatic activity to be detectable. Presence/absence values were calculated from 405 Coliform and 315 *E. coli* inoculated food samples during AOAC Validation study. Accordingly, presence/absence RLU thresholds are shown in Table 2 below for EnSURE. EnSURE Touch makes interpretation easier because its software does the calculation for you using data generated from the AOAC Validation Studies as well as additional internal testing.

Table 2: Potential Presence/absence threshold values for qualitative measurements

Result	EnSURE™
Absence	0
Caution	1
Presence	≥2

Most Coliform & *E. coli* bacteria at low inoculum levels incubated for 8 hours will produce sufficient enzyme activity and will be detected. Extending incubation beyond 8 hours may increase sensitivity/inclusivity especially if the organisms were stressed.

Results showing small RLU outputs (e.g., 3 or 4) are indicative of low-level contamination. RLU output will gradually increase with extended incubation of Detection Device at 37°C for 10 minutes or more. Conversely, samples without contamination will show no increase in RLU output (see Table 3). This provides a greater assurance to interpretation of results.

Table 3: Extended incubation

Instrument	1 st Result (10-minute incubation)	2 nd Result Extended Incubation	Result
EnSURE™	4 RLU	4 RLU	Negative
EnSURE™	4 RLU	10 RLU	Positive

AOAC Validation:

Foods tested under AOAC Research Institute *Performance Tested Methods*SM validation are listed in Table 4 below.

Table 4: Validated matrices

Coliform 6-hour assay Quantitative	<i>E. coli</i> 6-hour assay Quantitative	Coliform 8-hour assay Qualitative	<i>E. coli</i> 8-hour assay Qualitative
Ground Beef*	Ground Beef*	Ground Beef*	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	—	—
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	—

Inclusivity / Exclusivity:

Data from inclusivity and exclusivity studies demonstrate test performance based on organism detectability.

- Inclusivity describes detection of bacteria in Coliform and *E. coli* group; exclusivity describes the ability to exclude other bacteria that are not Coliform or *E. coli*, even at high inoculation levels.
- MicroSnap™ correctly measures all bacteria tested at required target level of 1,000 CFU/mL. A full list of both inclusive and exclusive organisms can be obtained from Hygiena™.
- MicroSnap™ also gives a very high probability of detection even at low level microorganism counts of 10-100 CFU/mL. (See Table 5 below). Sensitivity (<95%) reflects lowest inoculum levels and not failure to detect organisms.

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Table 5: Probability of detection at low level contamination (10-100 CFU/mL)

	8-hour Coliform %		8-hour <i>E. coli</i> %	
	EnSURE Touch ^A	EnSURE ^B	EnSURE Touch ^A	EnSURE ^B
Sensitivity	96	96	100	100
Specificity	100	100	100	100
PPV	100	100	100	100
NPV	100	100	100	100
Accuracy	100	100	100	100

A – EnSURE™ Touch Inclusivity data is derived from independent study at Campden Food Laboratories (n = 45 strains)

B – EnSURE Inclusivity data is derived from independent study at Campden Food Laboratories (n = 45 strains)

Calibration & Controls:

It is advisable to run positive and negative controls according to Good Laboratory Practice. Hygiena™ offers the following controls:

- Calibration Control Kit (Part # PCD4000)
- CalCheck (Part # CAL)

Sample Effects:

Samples Containing Natural Enzymes:

- Depending on enzyme levels in some sample types (e.g. fermented dairy products and cheeses, 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 Hygiena™ 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.

Cross-Reactivity of Results:

- Due to limitations of chromogenic media technologies, some strains of *Shigella sonnei* may be detected. *Shigella sonnei* is genetically very similar to generic *E. coli* and the subtle differences may not be identified via biochemical/chromogenic detection.
- Additionally, strains of *Hafnia alvei* may not be detected during Coliform detection and enumeration due to the limitations of current biochemical/chromogenic technologies

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 Hygiena's R&D team 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™ has not been tested with all possible food products, food processes, testing protocols or with all possible strains of the Coliform family.
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.

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Hygiena Liability:

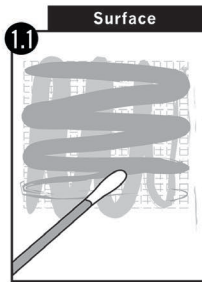
As with any culture medium, MicroSnap™ Coliform and *E. coli* results do not constitute a guarantee of quality of food, beverage products or processes that are tested with these devices. Hygiena will not be liable to user or others for any loss or damage, whether direct or indirect, incidental or consequential 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:

If more information or support are required, please visit www.hygiena.com/contact-us/

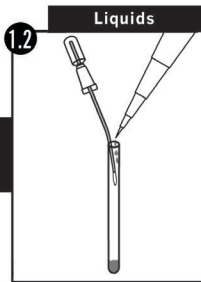
Hygiena™ – Americas Phone : 805.388.8007 Fax : 805.388.5531 Email : info@hygiena.com	Hygiena™ - International Phone : +44 1923 818821 Fax : +44 1923 818825 Email : enquiries@hygiena.com
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Step 1: Incubation of Sample



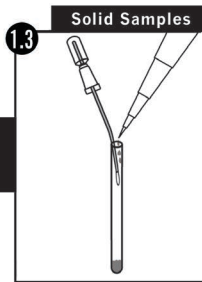
1.1 Surface: Swab 10x10cm area with Incubation Device.

or

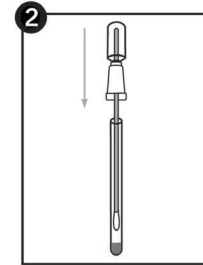


1.2. Liquids: Add 1 mL beverage or water sample directly to Incubation Device.

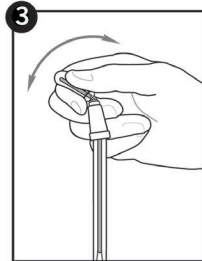
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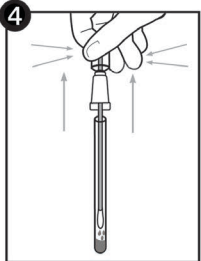
1.3. Solid Samples: Add 1mL 10% w/v suspension of solid samples directly to Incubation Device.



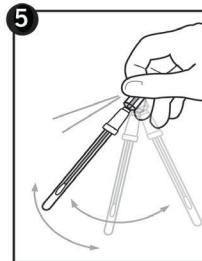
2. Reinsert Snap-Valve bulb into swab tube.



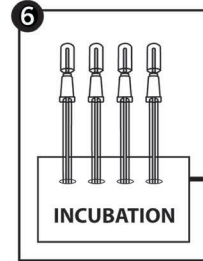
3. Activate the device. Bend bulb forward and backward to break Snap-Valve.



4. Lift bulb up (about 1–2") and squeeze bulb to release liquid into tube. Release pressure from bulb (bulb is like a dropper bulb) and replace bulb in tube. Most liquid should be in bottom of tube. **Incubation**



5. Shake tube gently to mix sample with liquid.

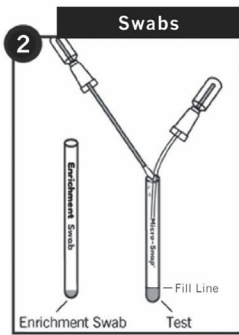


6. Incubate at 37°±0.5°C for 6 hours for quantitative measurement or 8 hours for qualitative measurement. This is the enriched sample. Proceed to Step 2.

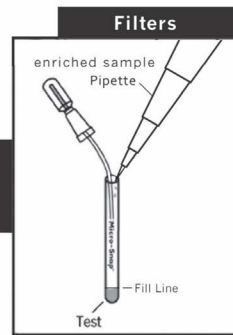
Step 2: Detection



1. Tap Detection Device on palm of hand 5 times to bring liquid in tube to bottom of tube.

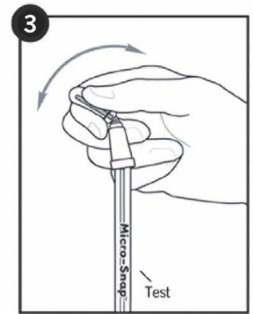


2.1: Swabs: Aseptically transfer 0.1mL (3 drops or to fill line) enriched sample from Incubation Device to Coliform Detection Device.

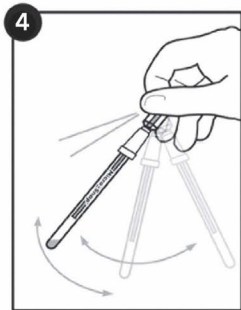


2.2: Filters: Aseptically transfer 0.1mL (3 drops or to fill line) enriched sample from Filtration /Petri dish to Coliform Detection Device.

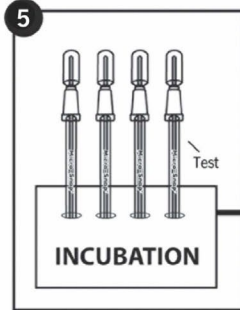
or



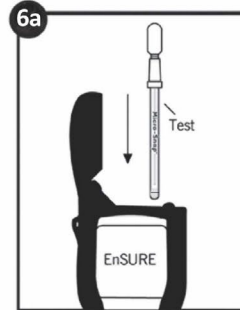
3. Activate Coliform Detection Device by bending bulb forward and backward, breaking Snap-Valve. Squeeze bulb three times to release liquid into tube.



4. Shake tube for 2 seconds to mix sample in liquid.



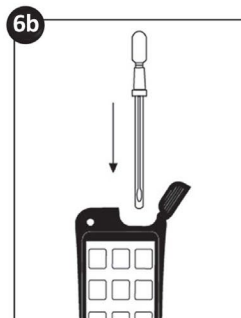
5. Incubate Coliform Detection Device for 10± 0.2 minutes at 37°± 0.5°C.



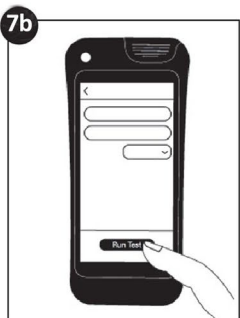
6a. For EnSURE™ Touch, skip to 6b. On EnSURE™, insert Detection Device and press "OK" to initiate measurement.



7a. Record RLU results and refer to the Results Interpretation Table for the conversion. If a positive result is obtained for Coliform, presence of *E. coli* can be verified using *E. coli* Detection Device. Repeat measurement procedure above using another aliquot sample from same sample.



6b. On the EnSURE™ Touch, open the MicroSnap™ application and select Quick Test or Samples depending on if you are performing a quick test or you are testing a sample which you previously programmed into EnSURE™ Touch.



7b. EnSURE™ Touch automatically saves your results. If a positive result is obtained for Coliform, presence of *E. coli* can be verified using *E. coli* Detection Device. Repeat measurement procedure above using another aliquot sample from same sample. 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. Find out more at www.hygiena.com/suretrendcloud.

Instructional videos:
www.youtube.com/HygienaTV
www.vimeo.com/HygienaTV