Directions for Use
MicroSnap – Coliform and *E. coli*

Part Numbers:
- MicroSnap Enrichment Device for environmental surfaces, liquids and food suspensions (Part # MS1-CEC)
- MicroSnap Enrichment Broth for filterable liquid samples (Part # MS1-CEC-BROTH-2ML)
- MicroSnap Coliform Detection Device (Part # MS2-COLIFORM)
- MicroSnap *E. coli* Detection Device (Part # MS2-ECOLI)

Description / Intended Use:
The MicroSnap test platform is a rapid bioluminogenic method for detection and enumeration of specific organisms. MS2-COLIFORM & MS2-ECOLI tests are designed to specifically detect Coliform and *Escherichia coli* in less than 8 hours.

Tests 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 small portable luminometer. In Step 1 (enrichment), sample is incubated in growth media in order to increase the number of bacteria. As the number of bacteria increase, more of the diagnostic enzymes are created (beta-galactosidase and beta-glucuronidase), which are required for the bioluminogenic reaction. Incubation time of sample is determined by the level of sensitivity required. After incubation, a small amount of sample is transferred to Detection Device. In Step 2, Detection Device is activated and incubated for 10 minutes. At this time, a specific substrate reacts with diagnostic enzymes to produce light. Light is measured in a luminometer in seconds. Light output is directly proportional to initial starting inoculum.

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, potable water and beverages.

Required Materials (Not Provided):
- Diluents for product samples e.g.
  - Buffered Peptone Water
  - Maximum Recovery Diluent
  - Butterfields
  - Other validated diluents of user's choice
- Sterile 0.45µm filters, filtration apparatus, 47mm Petri dishes for large volume liquid samples only
- Incubator at 37°C ± 0.5°C
- EnSURE or SystemSURE Plus luminometer

Test Procedure:

Instructional Video: www.youtube.com/HygienaTV

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

Environmental surfaces and product samples:
1) Collect sample and place in MicroSnap Enrichment Device (Part. # MS1-CEC).
   Samples can be:
   1.1 Surface – swab a 4 x 4 inches (10 x 10 cm) square area or for irregular surfaces swab as much of the surface as possible.
   1.2 Liquid - 1mL beverage or water samples added directly to Enrichment Device.
   1.3 Product - 1mL 10% w/v food homogenate added directly to Enrichment 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 piece back into swab tube. Device should look the same as it did when first pulled from the bag.
3) Activate device by bending bulb back and forth.
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. Place bulb into swab tube firmly to seal device.
5) Shake tube gently to mix sample and enrichment broth.
6) Incubate at 37° ± 0.5°C. For enumeration, incubate for 6 hours. For presence/absence, incubate for 8 hours.

For large volume filterable liquids:
1) Collect sample up to 100mL capacity and filter through 0.45 µm filter membrane with diameter 25mm or 47mm.
2) Aseptically remove filter and place into sterile 47mm Petri dish.
3) Aseptically add entire contents of Enrichment Broth (MS1-CEC-BROTH-2ML) vial to sterile Petri dish.
4) Incubate Petri dish at 37° ± 0.5°C. For enumeration, incubate for 6 hours. For presence/absence, incubate for 8 hours.

Step 2: Detection:
Detection procedure is described below and is also shown in Step 2 diagrams.
1) Allow MicroSnap Coliform or *E. coli* Detection Device 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 excess extractant liquid dispersed in tube to bottom of tube. Extractant is necessary to facilitate mixing of enriched sample with solution in tube.
2) Transfer enriched sample to Detection Device.
   2.1 Aseptically remove an aliquot of sample (optimum volume is 0.1mL, or 3 drops) from Enrichment Device and transfer to Detection Device. Enrichment Device can be used as a dropper tip for convenience. Squeeze and release bulb to mix and draw sample into bulb. Remove swab from tube and carefully dispense 3 drops (0.1mL) to fill line marked on bottom of Detection Device. Remaining enriched sample can be returned to Enrichment Device for additional testing.
   2.2 For filtered samples, aseptically pipette 0.1mL of incubated broth from Petri dish to Detection Device.
3) Activate Detection Device by bending bulb to break Snap-Valve. Squeeze bulb 3 times to release reagent.
4) Shake gently for 2 seconds to mix.
5) Incubate Detection Device for 10 minutes (± 0.2 min) at 37°± 0.5°C.
6) After 10 minutes of incubation, insert whole device into luminometer and close lid. Holding unit upright, press "OK" button to initiate measurement. Results will appear after 15 second count down.
7) Result will be displayed in RLU (Relative Light Units). Set thresholds on instrument that correspond to pass/fail levels deemed acceptable. See "Interpretation of Results" below for corresponding CFU levels.

Further Tests:
If a positive result is found using MicroSnap Coliform test, 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.

Interpretation of Results:
Results are displayed as Relative Light Units (RLU). Table1 shows equivalent colony forming unit (CFU) values to RLU. This will tell you how many Coliforms or *E. coli* CFU were present in original sample.

Hygiena's 2 luminometers approved for this test have different performance characteristics and sensitivities so RLU scales will differ accordingly (See Table 1). SystemSURE Plus and EnSURE luminometers have a 4-digit RLU output display and results ≥10,000 RLU will be outside display range.

Quantitative/Enumeration Measurements:
Incubation Time: 6 hours

RLU output is proportional to starting inoculum. 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%.

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

Estimated CFU	Equivalent RLU	
	SystemSURE Plus	EnSURE
<10	< 2	< 2
<20	< 3	< 4
<50	< 6	< 7
<100	< 8	< 12
<200	< 12	< 20
<500	< 25	< 35
<1,000	< 50	< 60
<5,000	< 85	< 180
<10,000	< 150	< 300

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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 enrichment 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.

Table 2: Presence/absence threshold values for qualitative measurements

Result	SystemSURE Plus	EnSURE
Absence	0	0
Caution	1	1
Presence	≥2	≥2

Most Coliform & *E. coli* bacteria at low inoculum levels incubated for 8 hours will produce sufficient enzyme activity and will be detected. Extending enrichment beyond 8 hours does not have any additional benefit for detection limits or sensitivity.

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	1st Result (10 minute incubation)	2nd 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	*E. coli* 6 hour assay Quantitative	Coliform 8 hour assay Qualitative	*E. coli* 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 Prawn	Raw Prawn	Raw Prawn	Raw Prawn
Mineral Water	Mineral Water	Mineral 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 detection of 10-100 CFU/mL. (See Table 5 below). Sensitivity (<95%) is a reflection of lowest inoculum levels and not failure to detect organisms.

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

	8 hour Coliform %		8 hour *E. coli* %	
	SystemSURE Plus[A]	EnSURE[B]	SystemSUREPlus[A]	EnSURE[B]
Sensitivity	94	96	88	100
Specificity	100	100	100	100
PPV	100	100	100	100
NPV	92	100	92	100
Accuracy	96	100	95	100

A – SystemSURE Plus data generated for AOAC validation study (n = 30 Coliform (all non *E.coli*) and n =30 *E.coli*
B – EnSURE Inclusivity data is derived from independent study at Campden Food Laboratories (n = 45 strains)

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)
- Coliform Positive Controls (Part # MS-PC-COLIFORM)

Sample Effects:
Some foods containing natural levels of specific enzymes may give elevated background levels that could be misinterpreted as false positives (e.g. some fermented dairy products and certain green leaf salad vegetables.) However these do not interfere with test performance and low levels of Coliform and *E. coli* are detectable above elevated background noise. For these foods, it is advisable to first check background levels by performing detection Step 2 before and after incubation. To avoid possibility of false positive results, threshold levels need to be adjusted to accommodate elevated background levels above the minimum detection threshold shown in Tables 1 & 2. For majority of foods, this is not a problem and this advice is purely cautionary.

Thick, opaque samples such as undiluted milk may affect output of light due to a blanching effect. Accordingly, further sample preparation and test validation may be required.

Some strains of *Shigella sonnei* may produce a false positive reaction which is also a limitation of chromogenic media, based on the same diagnostic principle. Strains of *Hafnia alvei* are also not detected.

Safety & Precautions:
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.

1. Devices are 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. Verify proper incubation temperature and time for the test application.

Storage & Shelf Life:
Bags of devices should be stored at 2 - 8°C.
Devices have a shelf life of 12 months. Expiration dates are printed on test devices.

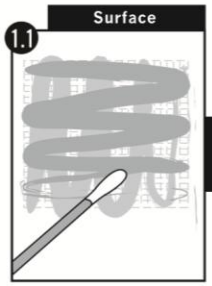
Caution & User Responsibility:
1. MicroSnap devices have not been tested with all possible food products, food processes, testing protocols or with all possible strains of the Coliform family.
2. Do not use this test for diagnosis of conditions in humans and animals.
3. No single culture medium will 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.

Hygiena Liability:
Hygiena will not be liable to user or others for any loss or damage whether direct or indirect, incidental or consequential from use of this device. 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 contact Customer Service for a Returned Goods authorization number.

Step: 1 Enrichment

MicroSnap™

Step 1: Environmental Surface Swabs, Liquids and Solid Samples



1.1 Surface: Swab 10x10cm area with Enrichment Device.

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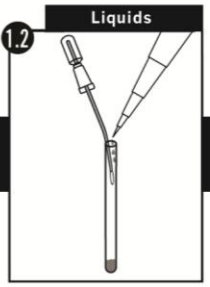

1.2. Liquids: Add 1 mL beverage or water sample directly to Enrichment Device.

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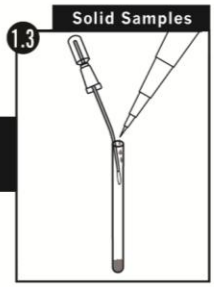
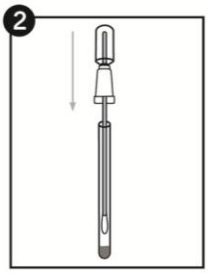
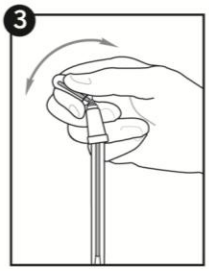


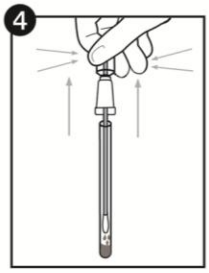
1.3. Solid Samples: Add 1mL10% w/v suspension of solid samples directly to Enrichment Device.



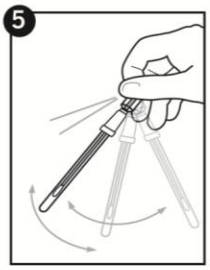
2. Reinsert Snap-Valve bulb into swab tube.



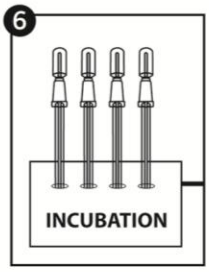
3. Activate the device. Bend bulb forward and backward to break Snap-Valve.



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.

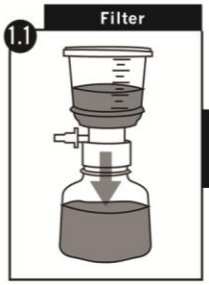


5. Shake tube gently to mix sample with liquid.



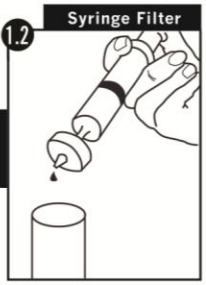
6. Incubate at 37° ± 0.5°C for 6 hours for quantitative measurement or 8 hours for qualitative measurement. This is the enriched sample. Proceed to Step 2.

Step 1: Large Volume Filterable Liquids

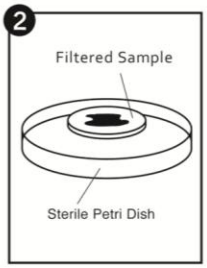


1.1 Filter: Filter sample through 0.45µm (micron) filter.

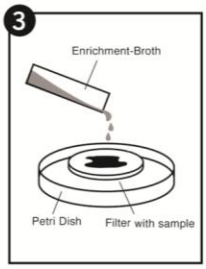
or



1.2. Syringe Filter: Filter sample through 0.45µm (micron) syringe filter.



2. Aseptically remove filter after filtration and place in sterile Petri dish.



3. Add 2mL Enrichment Broth to Petri dish.

4. 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.

Instructional video:
www.youtube.com/HygienaTV

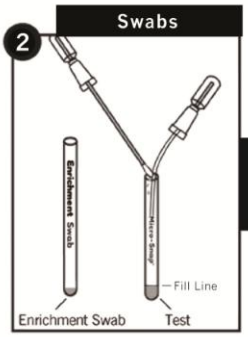
hygiena

Step: 2 Detection

MicroSnap™

Step 2: Detection

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



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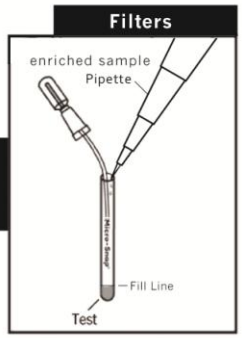
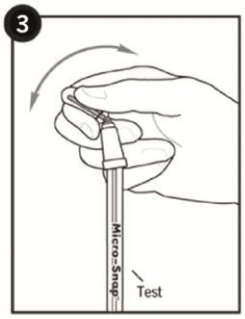
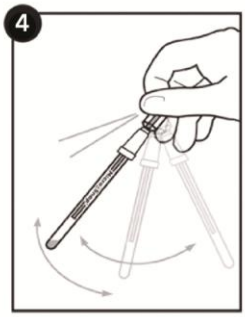


2.1: Swabs: Aseptically transfer 0.1mL (3 drops or to fill line) enriched sample from Enrichment Device to Coliform Detection Device.

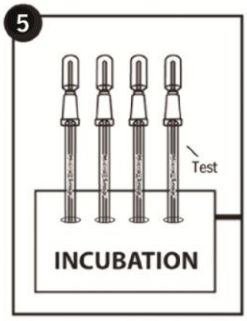
2.2: Filters: Aseptically transfer 0.1mL (3 drops or to fill line) enriched sample from Filtration /Petri dish to Coliform Detection Device.



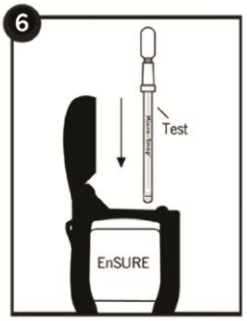
3. Activate Coliform Detection Device by bending bulb forward and backward, breaking Snap-Valve. Squeeze bulb three times to release liquid into tube.



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.



6. Insert Coliform Detection Device in luminometer and initiate measurement. Refer to Table 1 to interpret results.

7. If a positive result is obtained for Coliform, presence of *E. coli* can be verified using *E.coli* Detection Devic**e.** Repeat measurement procedure above using another aliquot sample from same enriched sample.

Instructional video:
www.youtube.com/HygienaTV