Directions for Use of
Micro-Snap – Rapid Determination of Coliform and E. coli

Catalog Number: MS-EC

Parts required:
- Micro-Snap Enrichment Swab device for environmental surfaces and food suspension (Part # ES-1000)
- Micro-Snap Enrichment Broth for filterable liquid samples (Part # EB-2000)
- Micro-Snap Coliform Test (Part # MS-COLIFORM)
- Micro-Snap E. coli Test (Part # MS-ECOLI)

Description/Intended use
Micro-Snap is a rapid bioluminogenic test method for the detection and enumeration of Coliform group giving results in less than 8 hours with confirmation of Escherichia coli. Micro-Snap consists of an enrichment device containing a non-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 followed by substrate development and detection steps. During incubation in enrichment broth, the number of bacteria is increased and their complement of inducible diagnostic enzymes is also amplified. Subsequently the action of these diagnostic enzymes on specific substrates in the detection device liberates light that is measured using a luminometer. The light output is directly proportional to the initial starting inoculum. The specific substrates will only be utilised by certain enzymes that are traditionally recognised as diagnostic of certain bacteria e.g. beta-Galactosidase for Coliform and beta-Glucuronidase for E. coli.

Micro-Snap can be used to test environmental surfaces, foodstuff, water and other filterable liquids. The test is intended to be used by an analyst with experience of microbiology and aseptic technique in a laboratory or other controlled facility.

Applicability
Micro-Snap will detect viable bacteria of the Coliform group and specifically E. coli. The results generated can be both qualitative (presence or absence) and quantitative to enumerate the bacteria present in the original sample. Occasionally some strains of Shigella sonnei may produce a false positive reaction which is also a feature of chromogenic media, based on the same diagnostic principle.

Micro-Snap has been validated for a wide range of foodstuff including representatives of the major food groups such as meat, dairy, fruit, vegetable, potable water and beverages as well as environmental surfaces. Occasionally some foodstuff containing high natural levels of specific enzymes may give high backgrounds starting levels e.g. some fermented dairy products and certain green leaf salad vegetables. However these do not interfere with the performance of the test and low levels of Coliform and E. coli are detectable above elevated background noise.

Material and reagents required but not provided
- Sample preparation equipment and diluents.
- Recommended Diluents for product samples:
  - Buffered Peptone Water
  - Maximum Recovery Diluent
  - Butterfields
  - Other validated diluents of users choice
- Sterile 0.45 μm filters and filtration apparatus
- Incubator at 37°C ± 1°C
- Luminometer
  - SystemSURE Plus (Hygiena), or
  - EnSURE (Hygiena), or
  - Pi 102 (Hygiena)

Test procedure

Step 1: Enrichment

The enrichment procedure for quantitative (enumeration) measurements is described as follows and is also shown in the Step 1 diagram.

For environmental surfaces and solid foodstuffs
A. Collect sample and place in the Micro-Snap Enrichment swab (Part. # ES-1000).

Samples can be of the following types:
- i. Surface Swabs (typically 4 x 4 inches; 10 x 10 cm) see diagram step1A1.
- ii. 1mL liquid food, beverage or water samples added directly to Micro-Snap Enrichment Swab (see diagram step 1A2)
- iii. 1mL 10% w/v food homogenate added directly to enrichment swab. The food homogenate is prepared using recommended diluents and standard microbiological procedures (see diagram step 1A3)

B. Re insert the Snap valve Bulb into swab tube (see diagram step 1B).

C. Activate device by breaking the snap valve by bending the bulb (see diagram step 1C)

Test procedure Continued

Step 1: Enrichment Continued

D. Squeeze the bulb to release the enrichment broth into swab tube by raising the bulb/swab assembly (about 1 – 2") and separating it from the swab tube to release the internal pressure because the bulb acts like a dropper bulb (or Pasteur pipette). Ensure most of the enrichment broth is in the bottom of the swab tube, replace bulb/swab assembly firmly to close the device (see diagram step 1D).

E. Shake the tube gently to mix sample and enrichment broth (see diagram step 1E).

F. Incubate at 37° ± 1°C for 4hrs (see diagram step 1F).

For large volume filterable liquids
G. Collect sample up to 100 ml capacity and filter through 0.45 μm filter membrane of diameter 25mm and/or 47mm (see diagram step 1G).

H. Aseptically remove the filter after filtration and place it in a sterile 47mm petridish (see diagram step 1H).

I. Add 2mL of Enrichment media from Enrichment Broth vial (EB-2000) to the petri-dish (see diagram step 1I).

J. The petri-dish is then incubated at 37° ± 1°C for 4 hours (see diagram step 1J).

For qualitative measurements a further 3 hours incubation is required such that a total incubation time of 7 hours is achieved. Longer periods of incubation are possible if required; however, this neither impairs the results nor does it confer any additional benefit in terms for detection limit or sensitivity

Step 2: Detection

The procedure for the detection process is described as follows and is also shown in the Step 2 diagram below:

A. i. Allow the Micro-Snap Coliform Test (MS-COLIFORM) to equilibrate to room temperature (10 minutes).
 ii. Shake the test device by tapping on the palm of your hand 5 times (to bring the droplets of liquid dispersed in the tube to the bottom of the tube); prior to adding the Enriched sample to the tube. This will facilitate the mixing of the Enriched sample with the solution in the tube). See diagram step 2A.

B. Transfer enriched sample to the Micro-Snap Coliform Test.

i. Aseptically remove an aliquot of the sample (optimum volume is 0.1ml, (or 2-3 drops) from the Micro-Snap Enrichment Swab and transfer it to the Micro-Snap Coliform Test (MS-COLIFORM). The Enrichment Swab can be used as a Pasteur pipette for convenience. Squeeze and release the bulb to mix and withdraw the sample into the bulb. Remove the swab from the tube and carefully dispense 2-3 drops (~ 0.1ml) to the graduated fill line marked on the bottom of the Micro-Snap Coliform Test device (see diagram step 2B).

The remaining enrichment broth can be returned to the Enrichment Swab device for repeat testing, confirmation of E. coli using Micro-Snap E.coli Test (see diagram step 2B).

ii. For filtered samples, aseptically pipette 0.1ml of the incubated broth from the petri dish to the Micro-Snap Coliform test (MS-COLIFORM), (see diagram step 2B).

C. Activate Micro-Snap Coliform Test. Bend the bulb to break the snap valve. Squeeze bulb 3 times to release the reagent (see diagram step 2C).

D. Shake gently to mix (see diagram step 2D).

E. Incubate for 10 minutes (± 0.5 min) at 37°±1°C (see diagram step 2E).

F. Insert the whole device into the luminometer; close the lid and holding the unit upright press “OK” button to initiate the measurement. Results will appear after the 15 second count down (see diagram step 2F).

G. Read result as RLU from the display on luminometer and interpret the result as directed below.

Further testing

If a positive result is found using the Micro-Snap Coliform Test then the convention would be to confirm the presence or absence of E. coli from the sample. This can easily be done by re-testing the same Micro-Snap Enriched sample using the Micro-Snap E. coli Test (MS-ECOLI).
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Step 1: Environmental Surface Swabs, Liquids and Solid Samples

A1. Surface: Swab a 10x10cm area or larger depending on protocol with the Micro-Snap Enrichment Swab (ES-1000).

A2. Liquids: 1ml liquid food, beverage or water sample added directly to Micro-Snap Enrichment Swab.

A3. Solid Samples: 1ml 10% w/v suspension of solid samples added directly to Micro-Snap Enrichment Swab.

B. Reinsert Snap-Valve bulb into swab tube.

C. Activate the device. Bend bulb, snapping the Snap-Valve rod.

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

E. Shake the tube gently to mix sample in the liquid.

F. Incubate at 37° ± 1°C for 4 hours for a quantitative measurement or 7 hours for a qualitative measurement. Proceed to step 2.

G1. Filter: Filter sample through a 0.45µm (micron) filter.

G2. Syringe Filter: Filter sample through a 0.45µm (micron) syringe filter.

H. Aseptically remove the filter after filtration and place it in a sterile Petri Dish.

I. Add 2mL of Enrichment Broth (EB-2000) to the Petri Dish.

J. Incubate at 37° ± 1°C for 4 hours for a quantitative measurement or 7 hours for a qualitative measurement. Proceed to step 2.
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**Step 2: Measurement**

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**A1.** Allow Micro-Snap Coliform test (MS-COLIFORM) to equilibrate to room temperature.

**Aii.** Shake tube by tapping on the palm of your hand 5 times to bring liquid in tube to the bottom of the tube.

**B.** Swabs: Aseptically transfer 0.1mL (2 to 3 drops or to fill line) of enriched sample from Micro-Snap Enrichment Swab to Micro-Snap Coliform Test (MS-COLIFORM).

**Bii.** Filters: Aseptically transfer 0.1mL (2 to 3 drops or to fill line) enriched sample from Filtration/Petri Dish to Micro-Snap Coliform Test.

**C.** Activate Micro-Snap Coliform Test by breaking the snap valve with a snap and squeeze action.

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**D.** Shake the tube gently to mix sample in the liquid.

**E.** Incubate Micro-Snap Coliform Test for 10 minutes at 37°± 1°C.

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**F.** Insert Micro-Snap Coliform Test in a luminometer and initiate the measurement. Record the results as RLUs and refer to table to interpret the results.

**G.** When a positive result is obtained for Coliform the presence of E.coli can be verified using the Micro-Snap E.coli Test (MS-ECOLI) and repeating the measurement procedure steps A–G above using another aliquot sample from the same enriched sample.

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**Interpretation of results:**
The results displayed on the luminometer are displayed as Relative Light Units (RLU). The tables below shows the equivalent colony forming unit (cfu) values for RLU measurements obtained using the 3 luminometers in Table 1.

Compare the RLU output with the corresponding instrument in Table 1 – 3 and Figure 1 to obtain quantitative measurement of the Coliform numbers present.

The luminometers have different performance characteristics and sensitivities and their RLU scales differ accordingly. The SystemSURE Plus and EnSURE instruments have a 4-digit RLU output and results ≥10,000 RLU will be outside the range. The Pi 102 luminometer has a much bigger dynamic range (8 digits) and can display large RLU values. For the Micro-Snap application on the Pi 102, low RLU values of <300 are considered background noise that are not significant.

**For Quantitative measurements (4-hour enrichment):**
The RLU output is proportional to the starting inoculums and the corresponding bacteria equivalent numbers (expressed as colony forming units, cfu). Compare the RLU output with the corresponding instrument in Table 1. All instruments show very good correlation coefficients ($r^2 = 0.86 – 0.99$) i.e. an agreement between the two methods of >92%.

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**Table 1: Relationship between Micro-Snap RLU and numbers of Coliform in three instruments.**

| RLU values | Equivalent colony forming units (cfu) for
<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>≤10</td>
<td>≤60</td>
<td>≤40</td>
<td>Pi 102</td>
</tr>
<tr>
<td>30</td>
<td>250</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>1,250</td>
<td>300</td>
<td>Background signal</td>
</tr>
<tr>
<td>300</td>
<td>7,000</td>
<td>800</td>
<td>≤20</td>
</tr>
<tr>
<td>1,000</td>
<td>14,000</td>
<td>2,000</td>
<td>50</td>
</tr>
<tr>
<td>3,000</td>
<td>≥25,000</td>
<td>5,500</td>
<td>125</td>
</tr>
<tr>
<td>10,000</td>
<td>≥25,000</td>
<td>15,000</td>
<td>300</td>
</tr>
<tr>
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<td></td>
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<tr>
<td>1,000,000</td>
<td></td>
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<td>9000</td>
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</table>
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Figure 1: Relationship between RLU and number of Coliform (cfu/ml) detected in EnSURE instrument after 4 hours enrichment

For qualitative measurements (7 hour enrichment):
Qualitative (presence or 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, the inoculum for the enrichment test would either contain no bacteria or >10.

If grown for 7 hours at 37°C, then an inoculum of 1 cfu will create significant enzymatic activity to be detectable. The absence/presence values were calculated from 240 samples with statistical confidence levels >99.7%.

Accordingly, the presence/absence RLU thresholds for the 3 luminometers are shown in table 2 below.

Table 2: Presence/absence threshold value for qualitative measurements

<table>
<thead>
<tr>
<th>Result</th>
<th>SystemSURE Plus</th>
<th>EnSURE</th>
<th>Ph 102</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>0 – 9</td>
<td>0 – 9</td>
<td>0 – 309</td>
</tr>
<tr>
<td>Present</td>
<td>≥10</td>
<td>≥10</td>
<td>≥310</td>
</tr>
</tbody>
</table>

Confirmation of the presence of Escherichia coli:
The presence of E. coli in the enrichment is confirmed if a significant RLU signal in Micro-Snap E. coli Test (MS-ECOLI) is observed. The presence/absence RLU thresholds for the 3 luminometers are shown in table 3 below.

Table 3: Presence/absence threshold value for E. coli confirmation

<table>
<thead>
<tr>
<th>Result</th>
<th>SystemSURE Plus</th>
<th>EnSURE</th>
<th>Ph 102</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>0 – 9</td>
<td>0 – 9</td>
<td>0 – 309</td>
</tr>
<tr>
<td>Present</td>
<td>≥10</td>
<td>≥10</td>
<td>≥310</td>
</tr>
</tbody>
</table>

Controls
It is advisable to run positive and negative controls according to good laboratory practice.

Some foodstuff containing high natural levels of specific enzymes may give high backgrounds starting levels e.g. some fermented dairy products and certain green leaf salad vegetables. For these samples it is advisable to first check background levels by performing the detection step 2 before any incubation and again after 4 and 7 hours incubation to determine the rate of decay.

Compensation should be made for elevated background above the minimum detection threshold shown in Tables 1 – 3 in order to avoid the possibility of false positive results. For the majority of foodstuffs this is not a problem and this advice is purely cautionary.

Safety & Precautions:
Components of Micro-Snap devices do not pose any health risk when used correctly. Used devices that confirm positive results may be bio hazardous and should be disposed off safely in compliance with Good Laboratory Practice and Health and Safety regulations.
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. Ensure proper incubation temperature and time for the test application.
5. When activating devices, ensure that all the liquid in the bulb is transferred to the tube below.

Storage & shelf life:
Device have a shelf life of 12 months. Check expiration date on label.

Caution and user responsibility:
1. Micro-Snap 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 the 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 in selecting a test method to evaluate a sufficient number of samples of particular foods and microbial challenges to satisfy the user that the chosen method meets the user’s criteria.
5. As with any culture medium, Micro-Snap results do not constitute a guarantee of quality of food, beverage products or processes that are tested with these devices.
6. The user must train personnel in proper testing techniques.

Hygiene 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 Hygieny. Please call Customer Service for a Returned Goods authorization number.

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