Positive Control for MicroSnap Coliform and *E. coli*

Instructions Only – See Required Materials

Description / Intended Use:

Operation and performance of MicroSnap Coliform and *E. coli* can be verified with bacterial strains in easy-to-use and handle Vitroids™ (Sigma-Aldrich®). Vitroids are non-pathogenic and non-lethal individual dried discs containing known numbers of viably stressed organisms (10^4 CFU *E.coli*/ disc), providing a common stable reference material. Vitroids are manufactured under ISO Guide 34 and certified under ISO17025.

Note: Positive Control for MicroSnap Coliform and E. coli *is intended for confirmation of MicroSnap test device performance only. For instructions on using Positive Control for a side by side comparison of microbiological methods, or for spiking food samples, contact Hygiena for a recommended protocol.*

Required Materials (Not Provided):

- Sigma-Aldrich Escherichia coli ATCC® 11775™ Vitroids (Part No. RQC01708-10EA) available from www.sigmaaldrich.com
- MicroSnap Enrichment Device (Part No. MS1-CEC)
- MicroSnap Detection Device (Part No. MS2-COLIFORM and/or MS2-ECOLI)
- Sterile tweezers
- Incubator at 37 ± 0.5°C
- Hygiena Luminometer

Directions:

Instructional Video: www.youtube.com/HygienaTV

Step 1: Enrichment:

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

1. Allow Positive Control and Enrichment Device to equilibrate to room temperature (10 minutes at 22 – 26°C). Twist and remove Snap-Valve bulb to open Enrichment Device tube. Aseptically transfer 1 positive control disc to Enrichment Device tube by using sterile tweezers.
2. Re-insert Snap-Valve bulb into Enrichment Device tube.
3. Activate Enrichment 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 enrichment broth is in bottom of swab tube.
5. Re-attach swab back on to swab tube firmly to seal device.
6. Allow Positive Control to rehydrate at room temperature for 10 – 15 minutes.
7. Shake tube gently to mix Positive Control and enrichment broth.
8. Incubate for 8 hours at 37 ± 0.5°C.

Step 2: Detection:

Detection procedure is described below and is also shown in Step 2 diagrams.

1. Allow MicroSnap 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 extractant liquid to bottom of tube. Extractant is necessary to facilitate mixing of enriched sample with solution in tube.
2. Transfer enriched sample from Enrichment Device to Detection Device. Enrichment Swab can be used as a pipette for convenience.
   i. Squeeze and release Enrichment Device bulb to mix and draw sample into bulb.
   ii. Remove Enrichment swab from tube.
   iii. Open Detection Device by twisting and pulling to remove bulb. Set aside.
      i) Insert Enrichment swab tip into top of Detection Device tube (approximately 1 inch or 3 cm) and lightly squeeze Enrichment Device bulb to trickle 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.
   iv. Reassemble Detection Device to original state.
3. 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.
4. Shake gently to mix.
5. Incubate Detection Device for 10 minutes (± 0.2 min) at 37 ± 0.5°C.
6. Turn on EnSURE luminometer. If locations have been programmed, select location to be tested. After 10 minutes of incubation, immediately insert whole device into luminometer; close lid and 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). Refer to "Interpretation of Results" below for correlation.

Interpretation of Results:

Results are displayed as Relative Light Units (RLU). RLU output is proportional to the starting inoculums and corresponding bacteria equivalent numbers (expressed as Colony Forming Units, CFU). Table 1 shows expected RLU values for each MicroSnap Detection device and Hygiena luminometer. Compare RLU result to data in Table 1. If RLU result falls within RLU range in Table 1, MicroSnap test devices are functioning properly.

Table 1: Expected MicroSnap RLU Mean & Range at Approx. 10^4 CFU

10,000 (10^4) CFU	EnSURE RLU (ENSURE)	SystemSURE Plus RLU (SS3)
Coliform (MS-COLIFORM)	Mean 5,000 (3,500 – 6,500)	Mean 2,000 (1,400 – 2,600)
***E. coli* (MS-ECOLI)**	Mean 1,000 (750 – 1,250)	Mean 500 (400 – 600)

Calibration Control:

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

- Calibration Control Kit for EnSURE and/or SystemSURE Plus luminometers (Part # PCD4000)

Storage & Shelf Life:

- Store at 2 – 8°C.
- Check expiration date on label.

Disposal:

Positive Controls and used MicroSnap test devices can be disinfected by autoclaving or by soaking in 20% bleach for 1 hour, and then discarded as trash. Alternatively, Positive Controls and test devices may be disposed via a biohazard disposal facility.

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Safety & Precautions:

When used correctly under controlled laboratory conditions, MicroSnap Positive Control components do not pose any health risk, however Positive Controls should be regarded as a biohazard and should be disposed of safely in compliance with Good Laboratory Practice and Health and Safety Regulations.

- Detection Device is designed for a single use. Do not reuse.
- Do not use Positive Controls or MicroSnap devices after expiration date.
- Follow standard Good Microbiological Practices where appropriate.
- Ensure proper incubation temperature and time.
- Proper microbiological techniques and precautions should be used to minimize potential hazards and risks.
- Personnel must be trained in proper testing techniques.
- Due to the nature of microbiology, there will be a natural variation in numbers of organisms on each Positive Control. However, results will be consistent within a given range.
- For further safety instruction, refer to the Safety Data Sheet (SDS) available at www.sigmaaldrich.com catalog # RQC01708-10EA.

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.

Contact Information:

If more information is required, please visit us at www.hygiena.com or contact us at:

Hygiena - Americas
Phone: 805.388.8007
Email: info@hygiena.com

Hygiena - International
Phone: +44 (0)1923 818821
Email: enquiries@hygiena.com

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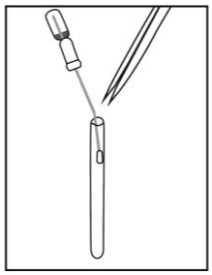
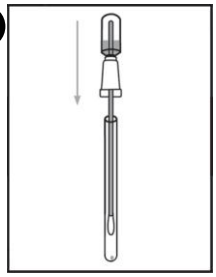
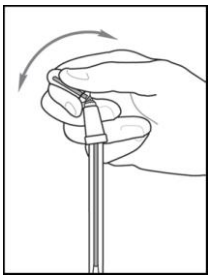
Step: 1 Enrichment of Positive Control



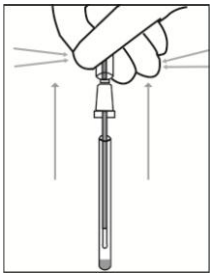
1. Add Positive Control to MicroSnap Enrichment Device tube.



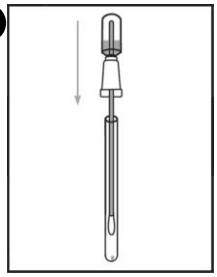
2. Reinsert Snap-Valve bulb into Enrichment Device tube.



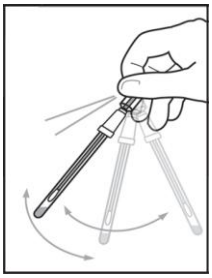
3. Activate device. Bend bulb forward and backward to break Snap-Valve.



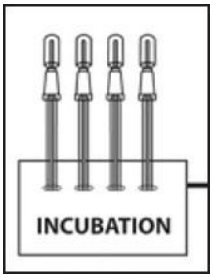
4. Lift bulb up and squeeze bulb to release liquid into tube.



5. Replace bulb in tube. Let stand for 10 – 15 minutes for the disc to dissolve.

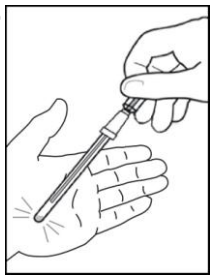


6. Shake tube gently to mix Positive Control in liquid completely.

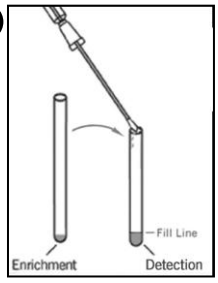


7. Incubate at 37 ± 0.5°C for 8 hours.

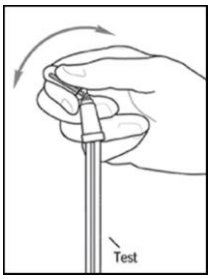
Step: 2 Detection & Measurement



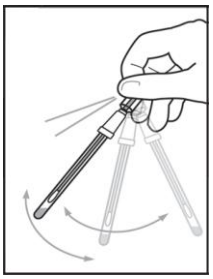
1. Shake Detection Device on palm of hand 5 times to bring liquid in tube to bottom of tube.



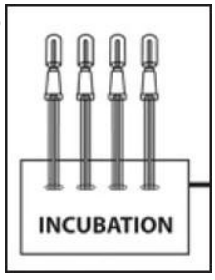
2. Aseptically transfer enriched sample from Enrichment Device to Detection Device.



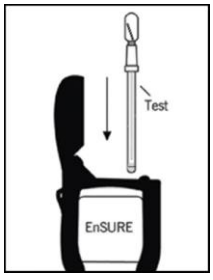
3. Activate Detection Device by bending bulb forward and backward, breaking Snap-Valve. Squeeze bulb three times to release liquid into tube.



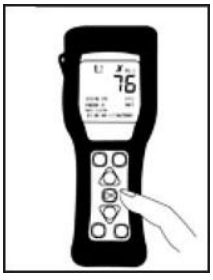
4. Shake tube gently to mix sample in liquid.



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



6. Insert Detection Device in luminometer and initiate measurement.



7. Record results as RLUs and refer to Table 1 to interpret results.