Directions for Use of
MicroSnap – Rapid Determination of Coliform and *E. coli*

Parts Numbers:
- MicroSnap Enrichment Swab device for environmental surfaces, liquids and food suspensions (Part # ES-1000)
- MicroSnap Enrichment Broth for filterable liquid samples (Part # EB-2000)
- MicroSnap Coliform Test (Part # MS-COLIFORM)
- MicroSnap *E. coli* Test (Part # MS-ECOLI)

Description / Intended Use

MicroSnap 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*. MicroSnap 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 unique substrates will only be utilized by specific enzymes within the bacteria e.g. beta-Galactosidase for Coliform and beta-Glucuronidase for *E. coli.*

MicroSnap 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 in microbiology and aseptic technique in a laboratory or other controlled facility.

Applicability

MicroSnap 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 limitation of chromogenic media, based on the same diagnostic principle. Strains of *Hafnia alvei* are also not detected.

MicroSnap 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. 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. Undiluted milk will cause blanching of the light this should be examined as part of a validation process.

Samples should be made into a 10% suspension where applicable; the sample size used during the validation was 50g.

Due to the nature of microbiology it should be noted that there will be a certain level of false positives and false negatives due to extraneous enzymatic activity in some samples. Due to the extreme sensitivity of the reaction if this is suspected then duplicate samples should be run to confirm.

More information on enzymatic activity in samples run during validations is available on request from Hygiena.

Material and reagents required but not provided

Equipment and diluents required for sample preparation.

Diluents for product samples e.g.

- Buffered Peptone Water
- Maximum Recovery Diluent
- Butterfields
- Other validated diluents of users choice

- Sterile 0.45 µm filters and filtration apparatus
- Incubator at 37ºC ± 1ºC
- Luminometer
  - SystemSURE Plus(Hygiena), or
  - EnSURE (Hygiena)

Test Procedure

<u>Step 1: Enrichment:</u> Sample, Activate and Incubate:

The enrichment procedure for quantitative (enumeration) measurements is described as follows and is also shown in the Step 1 diagrams. Hygiena recommend that the sample size adhere to standard methods, 50g of food per 450mL of diluent, other sample weights need to be validated by the user.

For environmental surfaces and solid foodstuffs

1) **Collect sample** and place in the MicroSnap Enrichment swab (Part. # ES-1000).
   Samples can be of the following types;
   1.1 Surface Swabs (typically 4 x 4 inches; 10 x 10 cm).
   1.2 1 mL beverage or water samples added directly to MicroSnap Enrichment Swab
   1.3 1mL 10% w/v food homogenate added directly to enrichment swab. The food homogenate is prepared using industry recommended diluents and standard microbiological procedures
2) Re insert the Snap valve bulb assembly into swab tube
3) **Activate** device by breaking the snap valve by bending the bulb
4) 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.
5) Shake the tube gently to mix sample and enrichment broth
6) **Incubate** at 37° ± 0.5°C for 6 hrs.
   This is referred to as the Enriched sample.

For large volume filterable liquids:

1) **Collect sample** up to 100 mL capacity and filter through 0.45 µm filter membrane of diameter 25mm and /or 47mm
2) Aseptically remove the filter after filtration and place it in a sterile 47mm petri-dish
3) Add 2mL of Enrichment media from Enrichment Broth vial (EB-2000) to the petri-dish
4) The petri-dish is then incubated at 37° ± 0.5°C for 6 hours.
   This is referred to as the Enriched sample.

For **qualitative measurements a further 2 hours incubation** is required such that a total incubation time of 8 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

<u>Step 2: Detection:</u> Transfer Enriched sample, Activate, Incubate and Measure.

The procedure for the detection process is described as follows and is also shown in the Step 2 diagrams;

1) Allow the Micro-Snap Coliform or *E. coli* Detection Test to equilibrate to room temperature (10 minutes).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).
2) **Transfer Enriched sample** to the Detection Device.
   2.1) Aseptically remove an aliquot of the sample (optimum volume is 0.1ml, (or 2-3 drops) from the Enrichment Swab and transfer it to the Detection Device. The Enrichment Swab can be used as a dropper tip 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 Detection device
   The remaining Enriched sample can be returned to the Enrichment Swab device for additional testing.
   2.2) for filtered samples, aseptically pipette 0.1mL of the incubated broth from the petri dish to the Detection Device.
3) **Activate** Detection Device. Bend the bulb to break the snap valve. Squeeze bulb 3 times to release the reagent
4) Shake gently to mix
5) **Incubate** for 10 minutes (± 0.2 min) at 37º±0.5ºC.
6) 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.
7) Result will be displayed in RLU (Relative Light Units). Set thresholds on the instrument that correspond to pass/fail levels deemed acceptable. See "Interpretation of results" section for corresponding CFU levels.

Further testing:

If a positive result is found using the MicroSnap Coliform Test then the convention would be to confirm the presence or absence of *E. coli* in the sample. This can easily be done by testing an aliquot of the same MicroSnap **Enriched sample** using the MicroSnap *E. coli* Test (MS-ECOLI). If an E.coli test is run solely then it should follow that confirmation should be run to determine E.coli positivity via standard methods.

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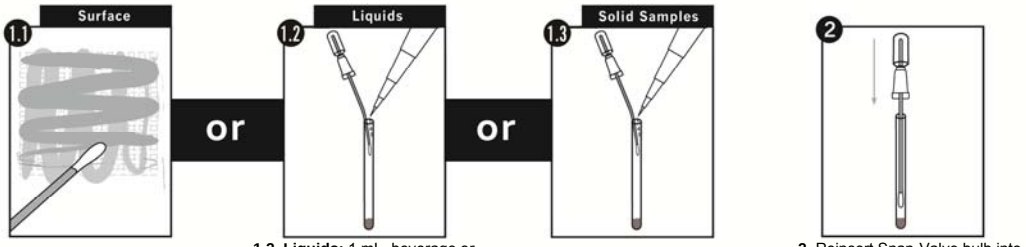
Step: 1 Enrichment

MicroSnap™

Step 1: Environmental Surface Swabs, Liquids and Solid Samples

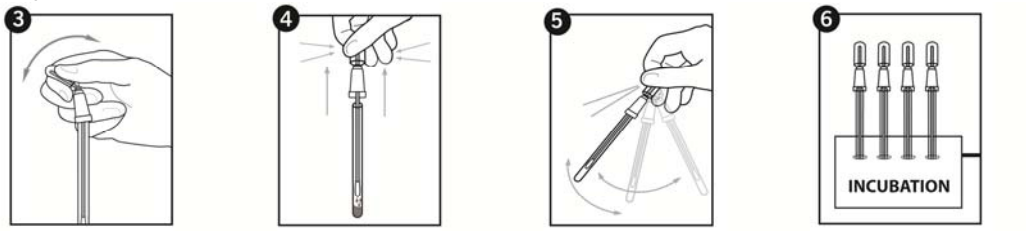


1.1 Surface: Swab a 10x10cm area or larger depending on protocol with the **MicroSnap Enrichment Swab (ES-1000).**

1.2. Liquids: 1 mL beverage or water sample added directly to **MicroSnap Enrichment Swab.**

1.3. Solid Samples: 1 mL10% w/v suspension of solid samples added directly to **MicroSnap Enrichment Swab.**

2. Reinsert Snap-Valve bulb into swab tube.



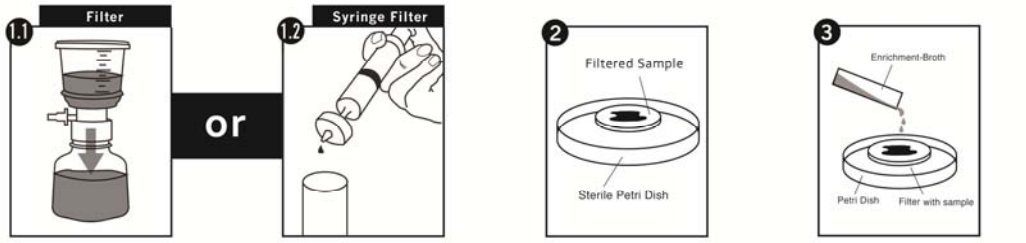
3. Activate the device. Bend bulb, snapping the Snap-Valve rod.

4. 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.

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

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

Step 1: Large Volume Filterable Liquids



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

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

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

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

4. Incubate at 37° ± 0.5°C for 6 hours for a quantitative measurement or 8 hours for a qualitative measurement. This is the Enriched sample.
Proceed to step 2.

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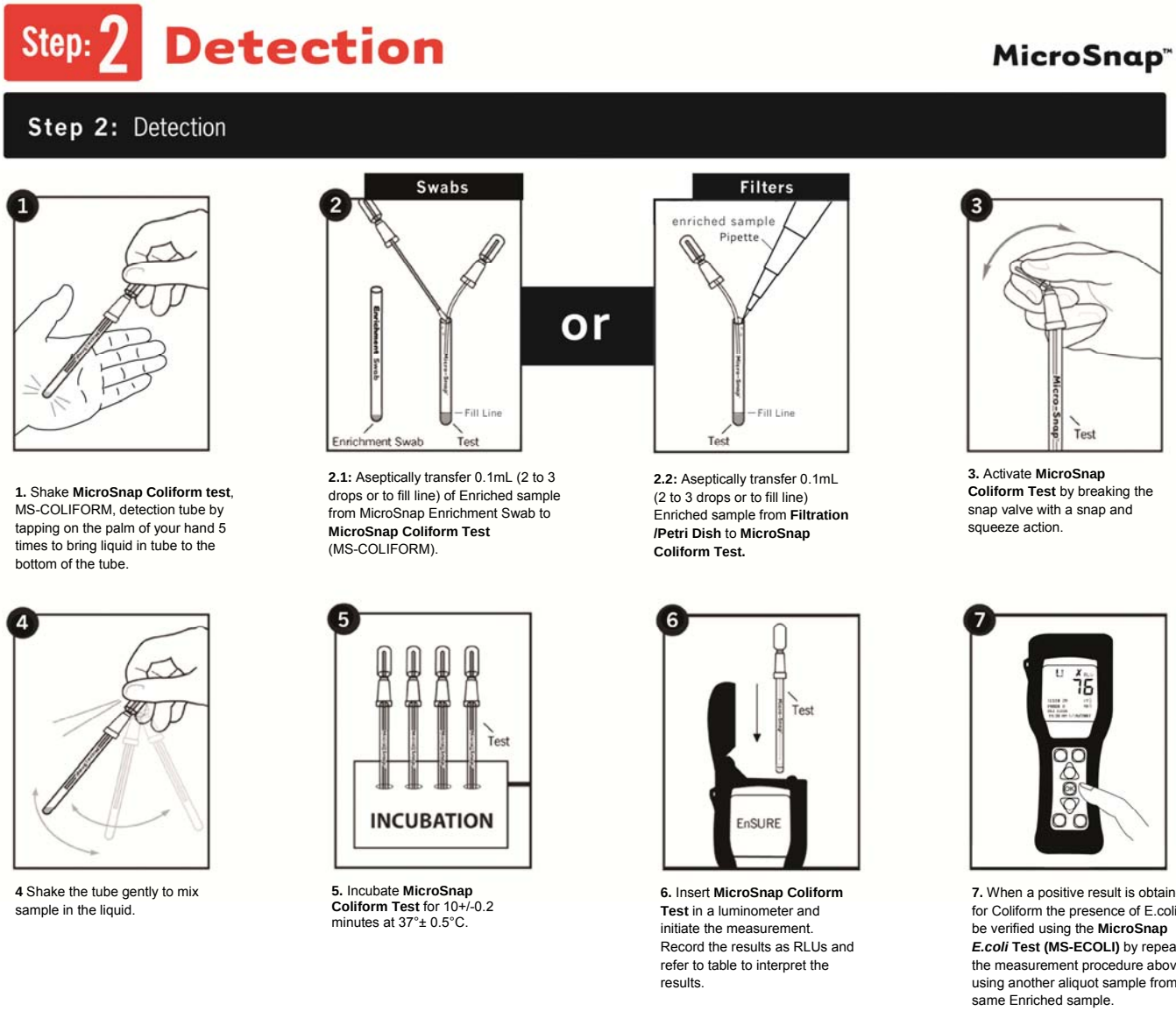

1. Shake **MicroSnap Coliform test**, MS-COLIFORM, detection tube by tapping on the palm of your hand 5 times to bring liquid in tube to the bottom of the tube.

2.1: Aseptically transfer 0.1mL (2 to 3 drops or to fill line) of Enriched sample from MicroSnap Enrichment Swab to **MicroSnap Coliform Test** (MS-COLIFORM).

2.2: Aseptically transfer 0.1mL (2 to 3 drops or to fill line) Enriched sample from **Filtration /Petri Dish** to **MicroSnap Coliform Test.**

3. Activate **MicroSnap Coliform Test** by breaking the snap valve with a snap and squeeze action.

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

5. Incubate **MicroSnap Coliform Test** for 10+/-0.2 minutes at 37°± 0.5°C.

6. Insert **MicroSnap Coliform Test** in a luminometer and initiate the measurement. Record the results as RLUs and refer to table to interpret the results.

7. When a positive result is obtained for Coliform the presence of E.coli can be verified using the **MicroSnap *E.coli* Test (MS-ECOLI)** by repeating the measurement procedure above using another aliquot sample from the same Enriched sample.

Interpretation of Results:

The results displayed on the luminometer are displayed as Relative Light Units (RLU). Table1 shows the equivalent colony forming unit (cfu) values in RLU.

Compare the RLU output with the corresponding CFU level. This will tell you how many Coliforms or E.coli CFU are present in the sample.

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 display and results ≥10,000 RLU will be outside the range.

Quantitative Measurements (6-hour enrichment required);

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; this data is derived from AOAC Validation study 2013. The percentage agreement between the traditional methods and MicroSnap method is greater than 92%

Table 1: **Relationship between Estimated CFU and Micro-Snap Coliform/ *E.coli* RLU.**

Estimated CFU	Equivalent RLU for 10 minutes assay on	
	SystemSURE Plus	EnSURE
<10	2	2
<20	3	4
<50	6	7
<100	8	12
<200	12	20
<500	25	35
<1000	50	60
<5000	85	180
<10,000	150	300

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Qualitative Measurements (8 hour Enrichment Required):

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 ≥1 CFU.

If grown for 8 hours at 37°C, an inoculum of 1 cfu will create significant enzymatic activity to be detectable. The absence/ presence values were calculated from 405 Coliform and 315 E.coli inoculated food samples during the AOAC Validation study. Accordingly, the presence/absence RLU thresholds are shown in Table 2 below.

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

Result	SystemSURE Plus	EnSURE
Absent	0	0
Caution	1	1
Present	≥2	≥2

Most bacteria at low inoculum or contamination levels will produce sufficient enzyme to be detectable after 10 minutes when they have been successfully incubated for 8 hours at 37ºC in the MicroSnap Enrichment device.

For reassurance of positive or negative results (e.g. products with elevated backgrounds), the assay time of the detection step can be increased beyond 10 minutes. A similar RLU output after a second extended period of incubation (e.g. 10 minutes) and subsequent repeat measurement of the same detection device, indicates a negative result, whereas an increase in RLU output indicates a positive test result (see Table 3).

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

Successful validation was performed and certified on the foods listed in Table 4 below.

Table 4: **Foods and Procedures certified under AOAC Research Institute *Performance Tested Methods*℠ Program**

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

Inclusivity describes the overall test performance based on a number of different organisms at low inoculum levels relevant to the intended use.
Inclusivity was 100% at the required target level of 1000 cfu/mL.

Table 5 **shows inclusivity at low level detection of 10 – 100 cfu/mL. Sensitivity (<95%) is a reflection of lowest inoculum levels and not the inability to detect the organisms.**

	8 hour Coliform %		8 hour E.coli %	
	SystemSURE Plus[A]	EnSURE [B]	SystemSURE Plus [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

Footnotes:
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)

Exclusivity Results

All gram negative and gram positive bacteria tested that did not belong to the Coliform or *E.coli* group were not detected. A full list of both inclusive and exclusive organisms can be obtained from Hygiena.

Controls:

It is advisable to run positive and negative controls according to good laboratory practice. Contact Hygiena for more information.

Specific Food Sample Effects

Some foodstuff containing high natural levels of specific enzymes may give high background starting levels e.g. some fermented dairy products and certain green leaf salad vegetables. For these food samples it is advisable to first check background levels by performing the detection Step 2 before and after incubation. Threshold levels need to be adjusted to accommodate elevated background levels above the minimum detection threshold shown in Tables 1 & 2 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. Undiluted milk may affect the output of light from the sample due to blanching this should be taken into account when validating this product.

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. Then, they can be placed in the trash. Alternatively, they can be taken to a biohazardous disposal facility.

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 the majority of liquid in the bulb is transferred to the tube below.

Storage & Shelf Life:

Bags of devices should be stored at 2 - 8°C.
Devices have a shelf life of 12 months.

Caution and 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 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, MicroSnap 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.

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 call 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

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