



InSite® *L. mono* Glo Environmental Surface Screening Test for *Listeria* spp. and *Listeria monocytogenes*

Product No: ILMG050 (50 tests)



Introduction

Description and Intended Use

InSite® *L. mono* Glo is a screening test for *Listeria* species and *Listeria monocytogenes* (*L. mono*), intended to be used for environmental monitoring in food processing environments after cleaning. The appearance of any grey or black color on the swab or in the medium is considered presumptive positive for *Listeria* species. Samples presumptive for *Listeria* species that exhibit green fluorescence under ultraviolet (UV 360–400 nm) light indicate the sample is presumptive positive for *L. mono*.

Scientific Principle

InSite *L. mono* Glo devices contain a proprietary formula of antibiotics, growth enhancers and color-changing compounds. Antibiotics inhibit most non-*Listeria* microorganisms, while growth enhancers provide recovery nutrients to support the growth of sub-lethally injured *Listeria*. β -glucosidase enzymes produced by *Listeria* species react with indicator compounds, changing the broth from yellow to black. Presumptive verification of *L. mono* is confirmed by green fluorescence under UV light due to the presence of the diagnostic enzyme, phospholipase C.

Intended User

Laboratory personnel trained in standard microbiological practices are qualified to use InSite *L. mono* Glo.

Required Materials (Not Provided)

- Dry Block Incubator (at 37 ± 1 °C) (Product No. INCUBATOR or INCUBATOR2)
- Block options for incubators:
 - 35 wells for swabs for INCUBATOR2 (Product No. IB001)
 - 12 wells for swabs for INCUBATOR (Product No. IB003)
- Self-sealing adhesive tape, plastic wrap or paraffin film
- Longwave (360–400 nm)* ultraviolet inspection lamp
- UV safety glasses

Important Tips Before Starting the Test

The foam tip swab is pre-moistened; condensation may be visible inside the swab tube—this is normal.

Use an aseptic technique: Do not touch the swab or the inside of the swab tube.

*AOAC RI PTM Studies were conducted using a wavelength of 395 nm



Test Procedures

1. Holding the swab tube firmly, twist and pull the top with the swab out of the swab tube. Thoroughly swab a standard 10 cm x 10 cm (4 in x 4 in) area of interest for a typical flat surface.

Important swabbing technique tips:

- a. Apply sufficient pressure to create flex in the swab shaft.
 - b. Swab in a crisscross pattern vertically, horizontally and diagonally in both directions.
 - c. Rotate the swab as the sample is being collected to ensure maximum sample pickup.
 - d. For irregular surfaces, ensure the swabbing technique remains consistent for each test and swab a large enough area to collect a representative sample.
2. Place the swab back in the swab tube and close the device firmly.

Tip: Seal the device to avoid accidental spillage by wrapping the joint of the swab tube and bulb with adhesive tape or self-sealing wrap.

3. To activate the device, hold the swab tube firmly, and use your thumb and forefinger to break the Snap-Valve by bending the bulb forward and backward. Squeeze the bulb 3 to 4 times, expelling all the liquid into the tube.
4. Gently massage the bottom of the tube by squeezing the tube 3 times, then shake for 3 seconds. This will help release cells from the swab and displace air bubbles.
5. Incubate for 24–48 hours at 37 ± 1 °C. Observe the color change (Figure 2) and record the result. Results cannot be considered negative until after 48 hours of incubation.
6. To verify the presence of *L. mono* after the appearance of a grey/black color (swab or medium), shine UV light directly into the side of the bottom portion of the tube.

Warning: Protect skin and eyes from UV light (see [Safety and Precautions](#)).

Tips:

- UV light should not be applied when the media remains amber or yellow after 48 hours.
- If green fluorescence is visible (Figure 2), the sample is presumptive positive for *L. mono*.
- To improve the visibility of the fluorescence, reduce ambient light levels.
- If the fluorescence is unclear, invert the test device. Since the fluorescent compounds bind to the tube material, the interpretation of results is improved when no media is present in the viewing area at the bottom of the tube (Figure 2).

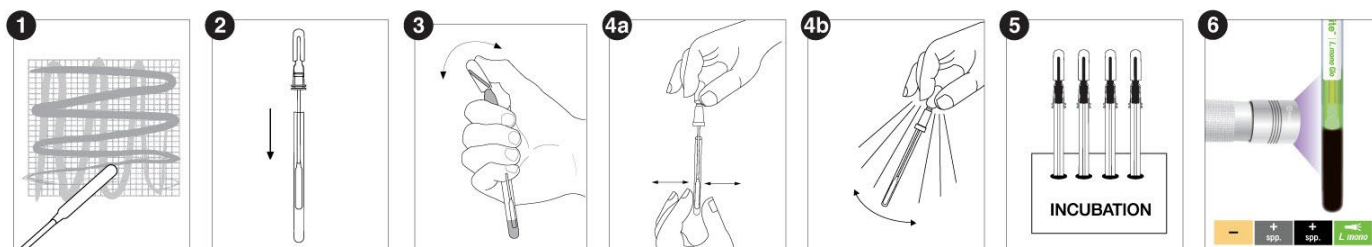


Figure 1. Workflow for the InSite *L. mono* Glo Device.



Additional Information

Interpretation of Results

For representative results, refer to Figure 2:

- No change in the media color after 48 hours indicates that the sample is negative for *Listeria* species.
- The appearance of any grey or black on the swab or in the medium is indicative of *Listeria* species.
- A grey/black color on the swab or in the medium with the presence of green fluorescence indicates that the sample is presumptive positive for *L. mono*.

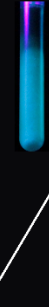
NATURAL LIGHT				UV LIGHT	
NEGATIVE	<i>LISTERIA</i> SPP.	<i>LISTERIA</i> SPP.	<i>LISTERIA</i> SPP.	<i>LISTERIA</i> SPP.	<i>L. MONO</i>
—	+ spp.	+ spp.	+ spp.	+ spp.	+ <i>L. mono</i>
					
YELLOW	SPOTS	GREY	BLACK	NO FLUORESCENCE	GREEN FLUORESCENCE
	PRESUMPTIVE POSITIVE FOR <i>LISTERIA</i> SPP.			NEGATIVE FOR <i>L. MONO</i>	PRESUMPTIVE POSITIVE FOR <i>L. MONO</i>

Figure 2. Interpretation of Color Changes Under Natural or UV Light.

Listeria-like Organisms

In high numbers, certain bacteria, such as *Enterococcus* spp., can produce blackening of the media or swab. Detection of these “*Listeria*-like” organisms in the environment can indicate that improvements in cleaning and sanitation are needed and that conditions at the sample site may be conducive to the growth of *Listeria*. A higher rate of presumptive positives can be expected when testing highly contaminated surfaces such as floors and drains.

To verify the presence of *Listeria* in a presumptive positive sample, Hygiena recommends testing the incubated media from the InSite device with a more specific method (e.g., PCR, ELISA or lateral flow). If InSite devices need to be stored or shipped before verification testing, keep them at 2 to 8 °C (36 to 46 °F) for up to 72 hours before testing.

AOAC RI Performance Tested MethodsSM Certification*

The detection of *Listeria monocytogenes* and *Listeria* spp. (*L. innocua*, *L. fleischmanni*, *L. welshimeri*, *L. weihenstephanensis*, *L. ivanovii* and *L. seeligeri*) using the InSite *L. mono* Glo device has earned AOAC RI PTM Certification (License #061802) from the AOAC Research Institute.



Validation studies included swabs (4 in x 4 in) of environmental surfaces (plastic, ceramic and stainless steel) read at a wavelength of 395 nm.

* Performance Tested MethodsSM is a service mark of AOAC International.



Confirmation

Presumptive positive samples can be confirmed with Hygiena's BAX® System PCR or Real-Time PCR Assays for Genus *Listeria* or *Listeria monocytogenes*.

Presumptive positive samples also can be confirmed by an appropriate regional reference method, such as:

- US FDA Bacteriological Analytical Manual (BAM)
- USDA FSIS Microbiology Laboratory Guidebook (MLG)
- Health Canada Compendium of Analytical Methods
- International Organization for Standardization (ISO)

Any confirmatory results should be handled according to appropriate regulations.

Storage and Stability

- Store at 2 to 8 °C (36 to 46 °F).
- Do not use past the expiration date on the label.

Disposal

Disinfect before disposal. InSite devices can be disinfected by autoclaving, incinerating or bleaching (soak unsealed device in 20% bleach for 1 hour). Then, they can be placed in the trash. Alternatively, InSite devices may be discarded following local disposal procedures.

Safety and Precautions

- UV lamps and UV flashlights are eye and skin hazards. Do not use without appropriate protection. Always wear a lab coat, gloves and safety glasses.
- InSite devices are intended to be used on production and environmental surfaces after cleaning.
- Components of InSite devices do not pose any health risk when used correctly. Used devices should be considered a biohazard and should be disposed of safely in compliance with Good Laboratory Practice and Health and Safety Regulations (see disposal instructions above).
- *Listeria monocytogenes* is a human pathogen. When handling samples that possibly contain *L. mono*, extreme care should be taken to contain the incubated samples of InSite devices. Immuno-compromised individuals and pregnant women are particularly susceptible to exposure to *L. mono* and should not be allowed near testing.

Hygiena Liability

As with any culture medium-based test, InSite *L. mono* Glo results do not constitute a guarantee of quality of food, beverage products or processes tested with these devices. Hygiena will not be liable to the 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; contact Customer Service for a Returned Goods Authorization Number.

Contact Information

For more information, visit www.hygiena.com/contact. For technical support, visit www.hygiena.com/support.