

## Technical Bulletin: A Rapid Method to Confirm InSite *Listeria* Swab Results Using the Lateral Flow System

An internal evaluation was conducted to assess the utility and performance of using the Lateral Flow System for *Listeria* as a confirmation method for positive color changes observed with the InSite *Listeria* environmental test swabs. Representative target organisms were tested for inclusivity, and similarly non-target organisms were tested for exclusivity. The results demonstrate the ability of the Lateral Flow System to rapidly confirm presumptive positive results from InSite as either positive or negative.

### Introduction

In food processing environments, bacterial attachment to food contact surfaces can be a source of contamination to foods (1). Resistant pathogens such as *Listeria* can survive on a wide range of surfaces used in the food industry by forming biofilms (2, 3). *Listeria innocua* and *L. monocytogenes* are the most commonly isolated species in food production facilities (4). The InSite *Listeria* and InSite *L. mono* Glo Swab are screening tests intended to be used for environmental monitoring in food processing facilities after cleaning. These devices produce a blackening of the media if the sample is presumptive positive and require additional tests to confirm the presence of *Listeria*. InSite *L. mono* Glo contains an additional fluorogenic substrate that is diagnostic of pathogenicity. Both tests have previously been validated as an accurate way to detect the presence of *Listeria* species or *L. mono* from environmental surfaces with equivalence to the USDA reference method, and are certified by AOAC RI PTM (ref 121902 and 061802).

This study was designed to evaluate the use of the Lateral Flow System for *Listeria* as a simple rapid confirmation of presumptive positive results from the InSite *L. mono* swab.

### Sample Preparation and Enrichment

Thirty-six strains were selected from the Hygiena Diagnostics culture collection and grown overnight in BHI. Among these strains, 26 were *Listeria* species (*monocytogenes*, *innocua*, *ivanovii*, *seeligeri* and *welshimeri*) and 10 were non-*Listeria* strains. The non-*Listeria* strains were represented by *Enterococcus* and *Bacillus*, known to show a positive color change with the InSite *L. mono* Glo swabs.

InSite *L. mono* Glo swabs were inoculated with an aliquot of each overnight culture. The swab was then placed back in the tube and firmly closed. Each swab was activated by bending the bulb forward and backward to break the snap-valve. The bulb was squeezed 3-4 times to expel the liquid down the into the tube. The tubes were gently mixed by squeezing the bottom of the tube and incubated at 37°C for 24-48 hours.

### Method

Insite *L. mono* Glo Method – For each swab device (ILMG050), the enrichment medium was visually inspected for color change from yellow/amber to grey/black at 24 and 48 hours.

Lateral Flow System Method – For each swab, 400 µL was removed and transferred to a microcentrifuge tube. Tubes were placed into a boiling water bath for 10 minutes and

cooled to room temperature. A Lateral Flow System test strip (KIT2037) was inserted into the sample with the arrows facing downwards into the tube. The strips developed for 10 minutes before result interpretation.

## Results and Discussion

For inclusivity testing, the InSite *L. mono* Glo swabs demonstrated a positive color change for all 26 *Listeria* strains at 24 and 48 hours. The lateral flow system demonstrated a positive predictive value of 96% after 24

hours, by returning positive results for 25/26 *Listeria* strains at 24 hours, and 24/26 *Listeria* strains at 48 hours (Table 1).

For exclusivity testing, the InSite *L. mono* Glo swabs demonstrated a positive color change for 7/10 non-*Listeria* strains at 24 hours, and 9/10 non-*Listeria* strains at 48 hours. The lateral flow system returned negative results for all 10 strains at 24 and 48 hours (Table 2). The lateral flow system negative predictive value is 100% after both 24 and 48 hours.

**Table 1. Inclusivity Results for the InSite *L. mono* Glo Swab and Lateral Flow System**

Genus Species	Strain ID	24 hours		48 hours	
		InSite Color Change	Lateral Flow Strip	InSite Color Change	Lateral Flow Strip
<i>Listeria monocytogenes</i>	DD605	POS	POS	POS	POS
<i>Listeria monocytogenes</i>	DD647	POS	POS	POS	POS
<i>Listeria monocytogenes</i>	DD648	POS	POS	POS	POS
<i>Listeria monocytogenes</i>	DD1072	POS	POS	POS	POS
<i>Listeria monocytogenes</i>	DD1145	POS	POS	POS	POS
<i>Listeria monocytogenes</i>	DD1146	POS	POS	POS	POS
<i>Listeria monocytogenes</i>	DD1147	POS	POS	POS	POS
<i>Listeria monocytogenes</i>	DD1149	POS	POS	POS	POS
<i>Listeria monocytogenes</i>	DD5849	POS	POS	POS	POS
<i>Listeria monocytogenes</i>	DD5850	POS	POS	POS	POS
<i>Listeria innocua</i>	DD644	POS	POS	POS	POS
<i>Listeria innocua</i>	DD921	POS	POS	POS	POS
<i>Listeria innocua</i>	DD922	POS	POS	POS	POS
<i>Listeria innocua</i>	DD1063	POS	POS	POS	POS
<i>Listeria innocua</i>	DD1155	POS	POS	POS	POS
<i>Listeria innocua</i>	DD1157	POS	POS	POS	POS
<i>Listeria innocua</i>	DD1158	POS	POS	POS	POS
<i>Listeria innocua</i>	DD1159	POS	POS	POS	POS
<i>Listeria innocua</i>	DD1162	POS	POS	POS	POS
<i>Listeria innocua</i>	DD1163	POS	POS	POS	POS
<i>Listeria ivanovii</i>	DD1164	POS	POS	POS	neg <sup>1</sup>
<i>Listeria ivanovii</i>	DD1165	POS	neg <sup>1</sup>	POS	neg <sup>1</sup>
<i>Listeria seeligeri</i>	DD3331	POS	POS	POS	POS
<i>Listeria seeligeri</i>	DD3800	POS	POS	POS	POS
<i>Listeria welshimeri</i>	DD4096	POS	POS	POS	POS
<i>Listeria welshimeri</i>	DD5912	POS	POS	POS	POS

1. The two *Listeria ivanovii* strains that returned a negative response with the lateral flow test strip are suspected to have insufficient antigen presentation to initiate a positive response. These cultures are also from an internal culture collection in which the strains are frequently used. This may have caused these strains to become attenuated.

**Table 2. Exclusivity Results for the InSite L. mono Glo Swab and Lateral Flow System**

Genus Species	Strain ID	24 hours		48 hours	
		InSite Color Change	Lateral Flow Strip	InSite Color Change	Lateral Flow Strip
<i>Enterococcus faecalis</i>	DD3981	POS	neg	POS	neg
<i>Enterococcus faecalis</i>	DD902	POS	neg	POS	neg
<i>Enterococcus faecalis</i>	DD2421	POS	neg	POS	neg
<i>Enterococcus faecalis</i>	DD2554	POS	neg	POS	neg
<i>Enterococcus faecalis</i>	DD2624	POS	neg	POS	neg
<i>Bacillus pumilus</i>	DD13937	POS	neg	POS	neg
<i>Bacillus licheniformis</i>	DD13932	POS	neg	POS	neg
<i>Bacillus thuringiensis</i>	DD714	neg	neg	POS	neg
<i>Bacillus cereus</i>	DD715	neg	neg	POS	neg
<i>Bacillus amyloliquefaciens</i>	DD593	neg	neg	neg	neg

## Conclusions

The results of inclusivity testing demonstrate the ability of the Lateral Flow System for *Listeria* as an accurate rapid confirmation to the InSite L. mono Glo swab presumptive positive results. Furthermore, exclusivity testing of non-target organisms that demonstrated a presumptive positive result with the InSite L. mono Glo swab can be resolved as negative with the Lateral Flow System.

Overall, the results of this study demonstrate the combined ability of the InSite L. mono Glo environmental test swabs and the Lateral Flow System for *Listeria* to detect and confirm the presence of *Listeria* species while clearing non-*Listeria* organisms as confirmed negative.

## References

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