Hardy Diagnostics Pathfinder™ Listeria Broth - An Enhanced Environmental Screening Media for Surface Testing

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Overview

Listeria monocytogenes was first described in 1926 by Murray, Webb and Swann as a cause of human illness and death, particularly in immunocompromised individuals and pregnant women. [1,2] The first reported food-borne outbreak of listeriosis was in 1985. [3] *Listeria monocytogenes* is a Gram-positive rod that is a non-spore forming, facultative anaerobe. As of 2019, there were 20 identified *Listeria* species within the *Listeria* genus but *Listeria monocytogenes* is considered to be the major pathogen responsible for listeriosis in humans. *L. monocytogenes* has been isolated from a wide range of food items including uncooked meats, uncooked vegetables, apples and other fruits, pasteurized or unpasteurized milk, foods made from milk, and processed foods, as well as in soil, sewage, silage, and river water. [4] *Listeria* species can grow over a pH range of 5.0-9.6 and survive in food products with pH levels outside of that range. [5] Infections caused by other *Listeria spp*. are extremely rare and only found in severely immunocompromised individuals such as those who are infected with HIV. Due to its cause fatality rate (CFR) of roughly 20%, *Listeria monocytogenes* is considered a widespread problem in public health and the food industry. The principle route of transmission is often via the consumption of foodstuffs contaminated with *Listeria monocytogenes* where the bioburden of a 25g sample may be less than 100 CFU to be considered infectious.

Hardy Diagnostics Pathfinder™ Listeria Broth was designed as an environmental screening test for surface samples that is selective and differential for the isolation and presumptive identification of pathogenic *Listeria monocytogenes.* With the use of a fluorescent indicator that is detectable by UV light, the media allows for the confirmation of *Listeria spp.* and is selective against commonly found surface organisms by utilizing selective agents and a darkening indicator. Hardy Diagnostics Pathfinder™ Listeria Broth is recommended for screening environmental surfaces from food processing environments or food contact surfaces, before and after cleaning. The medium was validated using sterile Puritan Enviromax™ tubes and swabs presoaked with neutralizing broth for incubating test organisms and surface samples. This tube/swab combination was used for both its cleaning agent neutralizing ability and also its material construction which is important for interacting with the added fluorescent compound. Hardy Diagnostics Pathfinder™ Listeria Broth was also tested in parallel with a similar competitor media for a performance comparison. Another comparison involved the use of an inhibitory agent (component A) to determine if adding the agent would be detrimental or beneficial in the final formulation. The main difference between Hardy Diagnostics Pathfinder™ Listeria Broth and the competitor media is that Pathfinder™ Listeria Broth is able to confirm the presence of *Listeria* spp. and presumptively identify *Listeria monocytogenes,* while the competitor can only detect *Listeria* presumptively. Both products showed equal presumptive results and excellent performance, but only Hardy Diagnostics Pathfinder™ Listeria Broth was able to confirm the presence of *Listeria* spp.

Materials and Methods

Organisms used in this validation were either ATCC or FDA sourced. Organisms labeled with an H as the first letter were sourced from a partner company, which were originally received from the FDA. All *Listeria* and *Enterococcus* or other negative control organisms were incubated on blood agar (Hardy cat.no A10) for 24-48 hours prior to dilution with tryptic soy broth (Hardy cat.no K89). All *Listeria* spp. were diluted to 1-50 CFU while negative control organisms were diluted to 10^4-10^5 CFU. Colony counts were then confirmed using tryptic soy agar (Hardy cat.no G62).

Puritan Enviromax™ (Hardy cat.no 2588050PF) polypropylene tube and swabs are used and tested for two reasons: 1) a polypropylene tube is necessary for the test due to the fact that the included compound that reacts with expressed enzyme attaches to only polypropylene and polyethylene where the glow reaction occurs, and 2) when swabbing surfaces that have been cleaned with sterilizing agents, a swab that is soaked with neutralizing broth as a part of HACCP (Hazard Analysis Critical Control Point) systems and other environmental monitoring testing is recommended. In order to confirm the presence of *Listeria* spp., a UV lamp that is able to produce light at 365nm is required to visualize the glow on inverted tubes between 24-48h of incubation at 35°C. Prior to incubation, the media is stored under refrigeration and away from light in a polycarbonate tube or bottle where the compound remains soluble in the media and cannot attach.

Surface testing was conducted on stainless steel. Autoclaved (15 min at 120 °C) 4X4 stainless steel plates were inoculated with 0.25ml, corresponding to ≤10^3 CFU and ≤10^2 CFU of *L. monocytogenes* and *L. ivanovii.* Samples were spread over the entire surface with a sterile spreader, and allowed to dry for 24 hours at room temperature. Surface counts were estimated by plating the same volume of each dilution onto tryptic soy agar. Puritan Enviromax™ swabs in tubes were then used to swab the surfaces. The swabs were placed back in the tubes and 15ml of Pathfinder™ Listeria Broth was aseptically poured into the tubes and capped tightly. The tubes were inoculated at 35 °C for 48hrs.

To show that *Listeria* spp. can be successfully transferred from Pathfinder™ Listeria Broth, HardyCHROM™ Listeria (Hardy cat.no G317) was tested. HardyCHROM™ Listeria is a selective chromogenic medium recommended for the isolation, differentiation, and enumeration of *Listeria monocytogenes* from food and environmental samples by colony color and appearance. The ability to transfer cells from Puritan Enviromax™ to HardyCHROM™ Listeria or other selective agars demonstrates that pure colonies can be isolated for a final confirmatory step as needed (e.g. PCR, API, and Micro-ID).

Results

There were two reasons for conducting this study. The first reason was to determine how The Hardy Diagnostics Pathfinder™ Listeria Broth compared to a similar media type designed for surface testing and detection of *Listeria* spp. The second reason was to determine how the addition of a media component (component A) in the media affects growth performance, the reactive enzyme production in *Listeria* spp., and the inhibition of *Enterococcus* spp. The addition of component A was determined to be beneficial and it did not affect overall performance of the media, so it was added to the final formula.

Hardy Diagnostics Pathfinder™ Listeria Broth is able to select for *Listeria* spp. from common surface contaminants and is able to differentiate *Listeria* spp. from *Enterococcus* spp. that are able to grow in the media. A confirmatory positive *Listeria* spp. result will glow green under UV light in 24-48 hours @35°C in a polypropylene or polyethylene container. In our testing, sterile polypropylene Puritan Enviromax™ tubes with neutralizing buffer were used. If *Enterococcus* is present in large concentrations (≥100 CFU) in the sample, some species such as *E. faecium or E.faecalis,* that have some resistance to selective agents in the media , may produce darkening of the media by 48 hours. In comparison to the competitor media, Hardy Diagnostics Pathfinder™ Listeria Broth is equally capable of detecting *Listeria monocytogenes* when using only media darkening as a test result. Among all organisms tested, Hardy Diagnostics Pathfinder™ Listeria Broth detected 96.3% (26/27) *Listeria* spp. and 100% *L. monocytogenes* by glow. All non-target organisms tested were unable to produce a glow in both formulas tested. The addition or exclusion of component A did not affect the identification of *L. monocytogenes,* but was able to slow *Enterococcus* growth overall and inhibit the growth of 56% of *Enterococcus* isolates tested by 48 hours. At 24 hours, the formula using component A was able to inhibit 96% (26/27) of all *Enterococcus* tested, while allowing 77.8% (14/18) of *L. monocytogenes* tested to produce a positive result. 100% of all *L. monocytogenes* tested produced a positive result by 48 hours.
While testing, inoculums were plated on TSA for colony count confirmation. All inoculations of *L. monocytogenes* detected over 48 hours were confirmed to be 1-50 CFU, the LoD of the media.

A limited study based upon selected AOAC performance tested methods was conducted for surface sampling and recovery over a 48 hour period on stainless steel 4X4 surfaces. By 24 hours, all *Listeria spp*. tested showed some darkening of the media, and by 48h 100% of all *Listeria spp.* showed a full darkening reaction and also glowed (Table 4).

Following the same methods, additional testing involved exclusive (non-*Listeria* spp.) background organisms and inclusive (*Listeria* spp.) organisms with and without the addition of component A. A total of 25 ATCC and FDA *Listeria* strains, 13 non-*Listeria* ATCC strains, and 27 ATCC and clinical *Enterococcus* strains were tested against the media (Tables 1-3.2).

Pathfinder™ Listeria Broth was inoculated with *Listeria monocytogenes* (ATCC 15313 and ATCC 7644) and incubated for 48 hours, showing a full positive result. The media was allowed to sit at room temperature for five days prior to plating on HardyCHROM™ Listeria media for further confirmation. A 10µl loop was inserted into Puritan Enviromax™ tubes and streaked onto the plates. At 24 hours, both plates confirmed the presence of *L. monocytogenes,* showing substantial turquoise colony growth, each surrounded by an opaque white halo. The purpose of this test was to show that *Listeria* spp. are able to survive for an extended period in Pathfinder™ Listeria Broth while being transported for confirmatory testing. Furthermore, the media does not interfere with the growth on other media types nor expected chromogenic development in HardyCHROM™ Listeria media.

24 and 48 Hour Limit of Detection Results

Component A inhibits *Enterococcus* growth but was thought to possibly slow the growth of *Listeria* also, so it was important to verify that its addition was beneficial to the formulation by improving the results. A combination of ATCC *Listeria* spp. and FDA sourced *Listeria monocytogenes* strains were tested against the competitor media over a 24-48 hour period at 35°C with inoculations of inclusive organisms (*Listeria* spp.) at 1-50 CFU (Table 1 and Images 1-3).

Table 1: Limit of Detection for All *Listeria* spp.

Organisms	Dilution (CFU)	24 hours					48 hours				
		*PF	*PF Glow	PF, No A	PF, No A, Glow	Competitor	*PF	*PF Glow	PF, No A	PF No A Glow	Competitor
L.mono (7644) **	(1-50)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(+)
L.mono (7644) / 10X E.coli (25922)**	(1-50)/ 10^2	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(+)
L.mono (15313) / 10X E.coli (25922)	(1-50)/ 10^2	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L.mono (15313)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L.ivanovii (BAA-139)	(1-50)	(+)	(-)	(+)	(-)	(-)	(+)	(+)	(+)	(+)	(+)
L.seeligeri (35967)	(1-50)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(+)	(+)	(-)
L.welshimeri (35897)	(1-50)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(+)	(+)	(+)
L. innocua (33090)	(1-50)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(+)	(+)	(+)
Listeria ivanovii (19119-LM4)	(1-50)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(+)
Listeria grayi (25401)	(1-50)	(+)	(-)	(+)	(-)	(-)	(+)	(+)	(+)	(+)	(+)
Listeria welshimeri (43549)	(1-50)	(+)	(-)	(+)	(-)	(-)	(+)	(-)	(+)	(-)	(+)
Listeria innocua (51742)	(1-50)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(+)	(+)	(+)
Listeria ivanovii (49954)	(1-50)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(+)	(+)	(+)
L. mono (H8391)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H8391)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H8392)	(1-50)	(-)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H8393)	(1-50)	(-)	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H8401)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H8964)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H6629)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (BA-11)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (BA-29)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H6822)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H6721)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H8395)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H9097)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H1238)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
		24 Hour Observed/ Expected (%)					48 Hour Observed/Expected (%)				
		63	0	66.7	0	59.3	*100	77.8	*100	96.3	96.3

* Both PF formulas presumptively identified all *Listeria spp.* by the darkening agent in both PF formulas at 48h.
** ATCC 7644 produced a very weak glow in both PF formulas by 48h
(+) refers to a positive result, (-) refers to a negative result.

All 3 formulas were compared over a 24-48 hour period against the competitor media at 35°C with inoculations of *Listeria monocytogenes*. All Inoculation sizes were confirmed to be 1-50 CFU (Table 2 and Images 1-3).

Table 2: Limit of Detection for *L. monocytogenes*

Organisms	Dilution (CFU)	24 hours					48 hours				
		*PF	*PF Glow	PF, No A	PF, No A, Glow	Competitor	*PF	*PF Glow	PF, No A	PF, No A, Glow	Competitor
L.mono (7644)***	(1-50)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(+)
L.mono (7644) / 10X E.coli (25922)***	(1-50)/ 10^2	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(+)
L.mono (15313) / 10X E.coli (25922)	(1-50)/ 10^2	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L.mono (15313)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H8391)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H8391)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H8392)	(1-50)	(-)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H8393)	(1-50)	(-)	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H8401)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H8964)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H6629)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (BA-11)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (BA-29)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H6822)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H6721)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H8395)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H9097)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H1238)	(1-50)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
		24 Hour Observed/ Expected (%)					48 Hour Observed/Expected (%)				
		77.8	0	83.3	0	88.9	100*	100**	100*	100**	100

* Both PF formulas presumptively identified all *L. monocytogenes* strains by the darkening agent at 48h.
** Both PF formulas confirmed all *Listeria* spp. by glow at 48h.
*** ATCC 7644 produced a very weak glow in both PF formulas by 48h

(+) refers to a positive result, (-) refers to a negative result.

All 3 formulas were compared over a 24-48 hour period against the competitor media at 35°C with inoculations of common (non-*Enterococcus*) surface contaminants tested in selected performance tested methods. All Inoculation sizes were confirmed to be 1-50 CFU. See Table 3 and images 1-3.

Table 3: Non-Target Organisms

(non- *Enterococcus*)

Organisms	Dilution (CFU)	24 hours					48 hours				
		*PF	*PF Glow	PF, No A	PF, No A, Glow	Competitor	*PF	*PF Glow	PF, No A	PF ,No A, Glow	Competitor
S.agalactiae (12386)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
S.agalactiae (QOVH1))	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
S.aureus (6538)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
S.aureus (29213)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
P.mirabilis(ARUP)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
P.mirabilis(12453)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
S.sonnei (9290)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
E.coli (25922)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
E.coli (8739)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
S.pyogenes (19615)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
B.cereus (10876)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
B.subtilis (9372)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
K.pneumoniae (13883)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
		24 Hour Observed/ Expected (%)					48 Hour Observed/Expected (%)				
		100	100*	100	100*	100	100	100*	100	100*	100

*Non target organisms are not expected to glow
(+) refers to a positive result, (-) refers to a negative result.

All 3 formulas were compared over a 24-48 hour period against the competitor media at 35°C with inoculations of 27 in house *Enterococcus* strains. All Inoculation sizes were 10^4-10^5 CFU. See Table 3.2 and images 1-3.

Table 3.2: *Enterococcus* spp.

Organisms	Dilution (CFU)	24 hours					48 hours				
		*PF	*PF Glow	PF, No A	PF ,No A, Glow	Competitor	*PF	*PF Glow	PF. No A	PF ,No A, Glow	Competitor
E. faecalis (29212)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(+)	(-)	(+)
E. faecium (700221)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(+)	(-)	(+)
E.faecium (972164)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(+)	(-)	(+)
E. faecalis (VRE 2)	(10^4-10^5)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(+)	(-)	(-)
E. durans (VRE 5)	(10^4-10^5)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
E. hirae (VRE 10)	(10^4-10^5)	(-)	(-)	(+)	(-)	(-)	(+)	(-)	(-)	(-)	(-)
E. casseliflavus (55)	(10^4-10^5)	(-)	(-)	(+)	(-)	(-)	(+)	(-)	(-)	(-)	(-)
E. faecalis (55)	(10^4-10^5)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
E. faecalis (950320)	(10^4-10^5)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
E.faecalis (956213)	(10^4-10^5)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
E. faecalis (979151)	(10^4-10^5)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
E. faecium (725854)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
E. faecium (862618)	(10^4-10^5)	(-)	(-)	(+)	(-)	(-)	(+)	(-)	(-)	(-)	(-)
E. avium (792164)	(10^4-10^5)	(-)	(-)	(+)	(-)	(-)	(+)	(-)	(-)	(-)	(-)
E. casseliflavus (25788)	(10^4-10^5)	(-)	(-)	(+)	(-)	(-)	(+)	(-)	(-)	(-)	(-)
E. cecorum (BAA150)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
E. flavescens (49996)	(10^4-10^5)	(-)	(-)	(+)	(-)	(-)	(+)	(-)	(+)	(-)	(-)
E. dispar (51266)	(10^4-10^5)	(-)	(-)	(+)	(-)	(-)	(+)	(-)	(+)	(-)	(-)
E. columbae (BAA598)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
E. sulfureus (49903)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(-)
E. pseudoavium (49372)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
E. malordoratus (43197)	(10^4-10^5)	(-)	(-)	(+)	(-)	(+)	(+)	(-)	(+)	(-)	(+)
E.hirae (48315)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
E. saccharolyticus (43076)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
E. raffinosus (49464)	(10^4-10^5)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
E. durans (6056)	(10^4-10^5)	(+)	(-)	(+)	(-)	(-)	(+)	(-)	(+)	(-)	(+)
E. avium (14025)	(10^4-10^5)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(-)
		24 Hour Observed/ Expected (%)					48 Hour Observed/Expected (%)				
		96	100*	41	100*	100	56	100*	63	100*	81

*Enterococcus is not expected to glow

(+) refers to a positive result, (-) refers to a negative result.

24 and 48 Hour Stainless Steel Surface Testing Results

Surface testing on stainless steel included *L. ivanovii* and *L. monocytogenes.* Full AOAC testing may include stainless steel, concrete, plastic (polycarbonate), and ceramic tile. See Table 4 and images 1-2.

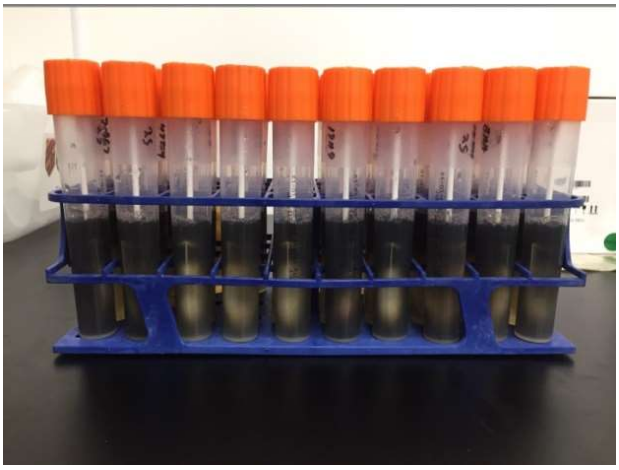
Table 4: Surface Testing On Stainless Steel

Organisms	Dilution (CFU)	24 hours					48 hours				
		*PF	*PF Glow	PF, No A	PF, No A, Glow	Competitor	*PF	*PF Glow	PF, No A	PF, No A, Glow	Competitor
L.mono (7644)	≤10^3	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L.mono (7644)	≤10^2	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L.mono (15313)	≤10^3	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
L.mono (15313)	≤10^2	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H8395)	≤10^3	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L. mono (H8395)	≤10^2	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L.ivanovii (BAA-139)	≤10^3	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
L.ivanovii (BAA-139)	≤10^2	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
		24 Hour Observed/ Expected (%)					48 Hour Observed/Expected (%)				
		100	0	100	25	100	100*	100**	100*	100**	100

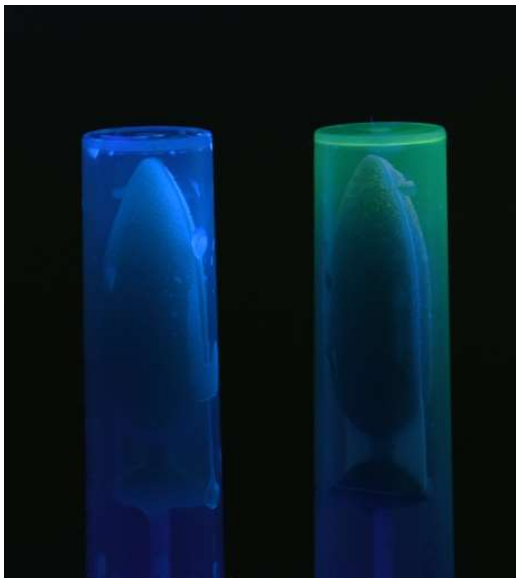
* Both PF formulas presumptively identified all *L. monocytogenes* strains by darkening agent reaction at 48h.
** Both PF formulas confirmed all *Listeria spp.* by glow at 48h.

(+) refers to a positive result, (-) refers to a negative result.

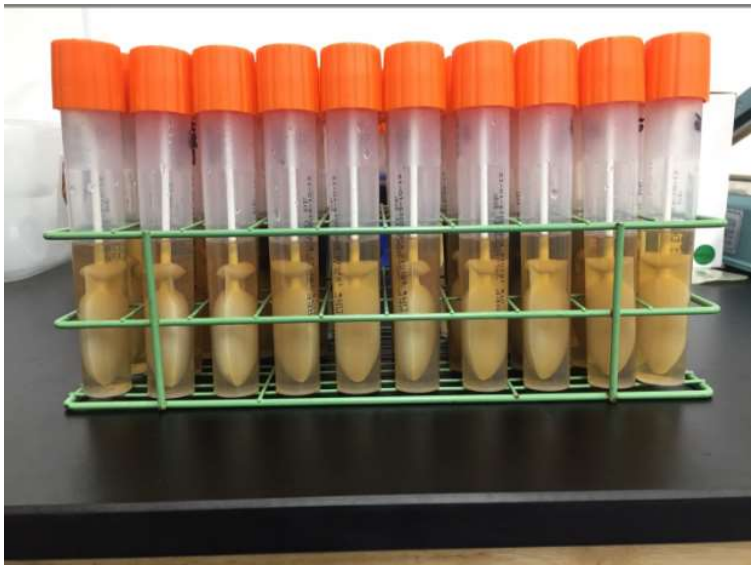
(Image 1) Pathfinder with component A and positive result for *Listeria* spp. after 48h incubation at 35°C in Puritan Enviromax™ tubes.



(Image 2) Pathfinder with formula component A inverted Puritan Enviromax™ tubes under UV light at 365nm after 48h incubation at 35°C. (Left) *Enterococcus spp*. (Right) *L. monocytogenes.*



(Image 3) Pathfinder with formula component A, negative result in Puritan Enviromax™ tubes.



Discussion

Hardy Diagnostics Pathfinder™ Listeria Broth is a selective and differential screening broth media for the isolation and presumptive identification of pathogenic *Listeria monocytogenes* and a confirmatory test for *Listeria* spp. from environmental surface samples. The patented fluorescent component of the Hardy Diagnostics Pathfinder™ Listeria Broth is not currently associated with any other known *Listeria* media to date. The common method for detection of *Listeria* spp. by the darkening agent alone is limited and can only be presumptive due to the possibility of *Enterococcus* contamination. Additional selective agents or further modifications to the media might be used to inhibit *Enterococcus*, but not without reduced performance in the growth of *Listeria,* so a moderate concentration of component A is a good compromise for slowing the growth of *Enterococcus* without slowing or inhibiting *Listeria* growth. A method for the confirmation of *Listeria* spp. becomes possible when the added fluorescent media component reacts with the specific reactive enzyme produced by *Listeria* spp. within 48h of growth in Puritan Enviromax ™ tubes. Because the fluorescent component in the media has an affinity for polypropylene, a green glow is visualized by shining a 365nm UV light on securely capped and inverted tubes. Since the media is highly selective against other common background flora, only *Listeria* spp. are able to grow and produce the enzyme necessary for a confirmatory result. As with other Listeria media, an enrichment broth may be used for isolation and to maximize recovery or shorten the time of detection, but is not necessary since the product is designed to be a single step system with built in enrichment and injury recovery. If additional enrichment is pursued prior to testing, users of Pathfinder Listeria Broth for food and environmental surveillance are recommended to follow the isolation and enrichment procedures outlined in the FDA's Bacteriological Analytical Methods (BAM) chapter 9.

The addition of component A to the Pathfinder medium improved the inhibition of resistant *Enterococcus* spp. and slowed its ability to darken the media. It was visually apparent that the formula without component A showed a much darker reaction and the formula with formula component A was lighter and showed greater inhibition by 24-48h. Hardy Diagnostics Pathfinder™ Listeria Broth was able to presumptively identify all *L. monocytogenes* tested and able to confirm the identity of *Listeria* spp. for most organisms tested. Though not all *Listeria* spp. glowed by 48h, all *Listeria* spp. produced a darkening reaction for a presumptive result. For presumptive detection of *Listeria* spp., Hardy Diagnostics Pathfinder™ Listeria Broth performed as well as the competitor media but was also able to confirm *Listeria* spp. by glow. All *L. monocytogenes* glowed by 48h and were confirmed as *Listeria* spp.

Additional testing demonstrated the ability to transfer from presumptively positive *L. monocytogenes* or confirmed *Listeria* spp. Puritan Enviromax ™ tubes to other media types was successful. ATCC 15313 and ATCC 7644 were both successfully transferred from Pathfinder™ Listeria Broth to HardyCHROM™ Listeria after a incubation period of five days at room temperature post confirmation of *Listeria* spp. The waiting period of five days at room temperature prior to plating demonstrates that *Listeria monocytogenes* will survive in the media for an extended period of time if confirmation testing is delayed. Transferring to plates or other selective media such as those described in FDA BAM and conducting PCR or other confirmatory analysis is possible and recommended.

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