FINAL REPORT
PDX-LIB Labor Saver
AOAC-RI Performance Tested Methods

Abstract

PDX-LIB Labor Saver method is derived from the previously approved PDX-LIB method (AOAC 040501) based on the fact that the industry wanted a "simpler" method. Derivation provided elimination of laborious handling and increased sampling size. Paradigm Diagnostics' *Listeria* Indicator Broth (PDX-LIB) is a specialty medium intended to be used as a screening tool for the detection of *Listeria* bacteria from environmental samples collected from food processing facilities. PDX-LIB is distinct because it is a single stage enrichment that can be used as a pre-screener for confirmatory tests. Interpretation of results is based on visible color change (darkening from yellow amber to brown black in color) after 30 to 48 hours of incubation at 37 °C. Performance of PDX-LIB Labor Saver has been tested in five different categories; inclusivity-exclusivity, method comparison, ruggedness lot to lot variability and shelf life. There were 67 *Listeria* and 37 non-*Listeria* species tested in the inclusivity-exclusivity studies. Inclusivity-exclusivity results suggested that all 67 species of *Listeria* tested gave positive results at less than 52 CFU. Only a few *Enterococcus* species gave false positive results at high cell counts. Method comparison studies were done for *Listeria monocytogenes* (Lm) in the presence of 1 log excess *E.coli* on sealed concrete, *L. innocua* (Li) on ceramic tile *L.ivanovii* (Liv) on stainless steel, and *L.welshmeri* (Lw) on plastic. Results of method comparison studies suggested that PDX-LIB Labor Saver performed better than the USDA method. Results of the ruggedness studies suggested deviations from the test parameters did not affect the test performance significantly. Lot-to-lot variability studies showed that there were no performance differences among three different production lots. PDX-LIB has been shown to pass QC criteria after 9 months of storage at refrigerated conditions.

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1 Scope of Method

PDX-LIB Labor Saver is a presumptive *Listeria* test that has been specifically designed to monitor presence of *Listeria* in environmental samples from the food processing environment. The test contains a liquid media formulated with antibiotics, growth enhancers, and color changing compounds specific to the growth of *Listeria* species. PDX-LIB Labor Saver has been vigorously tested not only at low levels of pure *Listeria* cultures (1-100 CFU/mL) but also in naturally contaminated environmental samples from the food industry during the initial developmental studies. Applicability of PDX-LIB Labor Saver is limited for selected common *Listeria* spp (Lm, Li, Liv, and Lw) on selected common surface types (sealed concrete, ceramic tile, stainless steel and plastic). In order to assess the performance characteristics, PDX-LIB has been tested in five different performance categories; 1) Inclusivity and exclusivity studies 2) Method comparison studies 3) Ruggedness studies 4) Lot to lot variability and 5) Shelf stability studies.

2 Definitions

2.1 Chi-squared (X^2) Value: The Chi-Square value for unpaired samples is frequently used in Official Methods to determine if two methods are equivalent. The formula is;

$$X^2 = N*\{[(N_{12})(N_{21})-(N_{11})(N_{22})]-N/2\}^2/(N_{12}+N_{11})(N_{12}+N_{22})(N_{22}+N_{21})(N_{11}+N_{21})$$

N_{12}= samples positive by Test method
N_{11}= samples positive by the reference method
N_{22}= samples negative by Test method
N_{21}= samples negative by the reference method

3 Principle:

The principle of the PDX-LIB is based on colorometric changes in enrichment medium in the presence of *Listeria spp* utilizing a unique blend of antibiotics, growth enhancers and *Listeria* specific color indicators working synergistically. A light brown to black color formation within 30 hours indicates presence of *Listeria spp* in an environmental samples. PDX-LIB has a limit of detection for most *Listeria spp* between (>1-100 CFU/mL) within 30 hours.

3.1 General Information

Listeria species are naturally found in the environment including animals, plants, soil, dust, and silage. *Listeria spp.* are gram-positive non-spore forming rods and have tumble motility. There are six different species of *Listeria*; *Listeria monocytogenes*,(Lm) *L. innocua*(Li), *L.ivanovii*(Liv), *L. welshmeri*(Lw), *L.grayi*(Lg), and *L.seeligeri*(Ls). Of these six species, *L.monocytogenes* and *L.ivanovii* are considered pathogens. *L. monocytogenes* is the most deadly of the pathogenic species to humans. *L. ivanovii* was

once considered to be a separate serotype of *L monocytogenes* until it was reclassified in 1984 as a new species (1). Because of this close connection to the human pathogen, *L. ivanovii* although more likely to cause sickness in animals, can be considered a rare but viable threat to humans (2).

For a non-spore forming bacterium, *L. monocytogenes* is relatively resistant to heating and drying and can multiply at temperatures as low as 3°C (3). Although it is able to withstand some heating and drying, it can still be injured during the course of sublethal heating, freezing, and exposure to detergents. It is the detection of this type of *Listeria* that is particularly important. Sub-lethally injured organisms can undergo repair in food product and cause illness to consumers (4).

Listeria spp. can typically be found in uncooked meats and vegetables, un-pasteurized milk, ice cream, soft cheeses like Swiss and Mexican, and ready to eat food products (RTE) (5). Animals carry these bacteria in their intestinal tract without showing signs of illness. These bacteria can then be transferred to meat as well as diary products during food processing.

Listeriosis is the disease caused by consuming food contaminated with *L. monocytogenes*. It is one of the leading causes of death not because it occurs often but because of the extremely high mortality rate (6). In the United States alone, there are over 2,500 reported cases of *Listeriosis* every year; of these there is approximately 500 deaths. Individuals who are immuno-compromised, young, old, or pregnant should be careful not to consume adulterated products. Symptoms may include fever, muscle aches, and diarrhea. If the nervous system becomes infected symptoms may include headache, stiff neck, confusion, loss of balance and convulsions. In pregnant women, *listeriosis* can produce flu like illness and can lead to miscarriage or stillbirth of the fetus (5). Because of the seriousness of the illness caused by *Listeria* contamination, government agencies have taken steps to reduce incidence of *Listeria* outbreaks including a "zero-tolerance" policy for *Listeria* in 25g samples of food product (1). However, several European countries instituted an action level for 100 CFU/g for *L.monocytogenes* (7). Furthermore according to Chen *et al*, 2003(8), foods containing low levels (<100 cfu/g) of *L.monocytogenes* pose very little risk.

Between January 2004 and April of 2004, there has been six recalls on various food products contaminated with *Listeria* (9). Each of these recalls cost companies a tremendous amount of money as well as reputation among consumers.

Current methods for enrichment and detection of *Listeria spp.* can take 3-5 days for presumptive results and an additional 2-3 days for confirmation. This method is time consuming and requires a great amount of work (3). It is also important to note that the detection of *Listeria* is limited by the abilities of the method. Detection and enrichment medias that rely heavily on selective agents may not be able to culture out sub-lethally injured *Listeria* organisms. Although these organisms occasionally do not show up in the culture method, they can persist in the food product and cause an outbreak to occur (4).

It is clear that a more rapid method with equal or better sensitivity is needed to control *Listeria* in the food industry.

4 Test Kit Information

4.1 Kit Name: PDX-LIB

4.2 Catalog Numbers: 25003-25, 25004-100, 25005-100, 25009-50

4.2.1 In the US and Canada

Paradigm Diagnostics, LLC
1360 University Ave. W, Suite 455
St. Paul, MN 55104
Web: www.pdx-inc.com

Hardy Diagnostics
Christopher A. Catani MT(ASCP), RM(ACM)
Director of Sales, Marketing & Technical Support

Ph: (800) 266-2222 Ext. 5696
Fx: (805) 614-9274
CataniC@HardyDiagnostics.com
www.HardyDiagnostics.com

Hardy Diagnostics
1430 West McCoy Lane
Santa Maria, CA 93455

4.2.2 New Zealand

Food Tech Solutions Ltd

Peter J. Bailey
Director of Sales and Marketing,
Technical Support

Ph: (64) 9 576 7326
Fax: (64) 9 576 7005
foodtech@ihug.co.nz
www.fts.co.nz

4.3 Test Kit Reagents

4.3.1 Aseptically filled 20 mL of PDX-LIB tubes

5 Additional Supplies and Reagents

5.1 Tecra Enviroswabs

5.2 MOX plates: Modified Oxford Agar plates for optional confirmation step

6 Apparatus

6.1 Incubator at 37 ºC (not included in the test kit)

7 Standard Reference Materials

All reference materials required by USDA document MLG 8.04 have been used as necessary (Source: http://www.fsis.usda.gov/Ophs/Microlab/Mlg_8_04.pdf)

8 Standard Solutions

Not applicable

9 Safety Precautions

PDX-LIB Labor Saver test should be done by personnel with basic microbiology laboratory training. Material Safety Data Sheets are available upon request. All materials used should be handled and disposed of as potentially infectious material. Autoclaving is the preferred method of disposal. If autoclaving is not available, disinfectant solutions should be used. *Listeria monocytogenes* is a dangerous human pathogen. When handling samples that possibly contain *L.monocytogenes*, extreme care should be taken to contain the samples and the enriched samples (presumptive positive tubes). Immuno-compromised individuals and pregnant women are particularly endangered by exposure to *L.monocytogenes* and should not be allowed in the vicinity of the testing.

10 General Preparation

The pure cultures were obtained from R-Tech Labs, Arden Hills, MN, American Typed Culture Collection (ATCC), Manassas, VA, Veterinary Medical Diagnostics Labs at University of Minnesota, St. Paul, MN, and Microbiology Labs of Dr. Diez and Dr. Feirtag at the University of Minnesota, St. Paul, MN. Original pure cultures were grown overnight in sterile Tryptic Soy Broth (TSB) at 32-37 ºC. In order to have reproducible cultures, 50 % glycerol stock solutions of cultures were prepared and stored in a freezer. Fifty percent glycerol stock cultures were prepared by diluting 500 µL of pure culture grown overnight with sterile 500 µL glycerol. Then 200 uL portions were filled into sterile 2 mL centrifuge tubes, capped and kept in a freezer at –15 ºC until the day before use. On the day before use, a loop-full of freezer-stored stock cultures were inoculated into 5 mL of sterile TSB, the same loop was streaked onto tryptic soy agar (TSA) containing ferric ammonium citrate and esculin (TSAIE). The TSB and TSAIE were incubated overnight at 37 ºC. TSAIE plates were checked for contamination, based on β-glucosidase activity and colony morphology. If the TSAIE plates suggested there was no apparent contamination, logarithmic level dilutions were made into sterile peptone solutions (0.1 % peptone in distilled water). Aliquots containing 100 µL of 6 and 7 log dilutions from overnight cultures were plated onto TSAIE for estimating the cell concentration (CFU/mL) and checked for contamination.

11 Sample Preparation

11.1 Sampling for AOAC-RI Guideline Studies:

Dilutions of pure cultures aforementioned were used for all AOAC-RI recommended studies, which were inclusivity-exclusivity, method comparison, ruggedness and lot to-lot variability and shelf stability studies.

12 Analysis

- Take an environmental sample following USDA recommendations for sampling. Return the sampling device back into the original sterile container.
- Aseptically add one unit (20 mL) of PDX-LIB media to the sterile container to fully submerge the applicator tip.
- Incubate in upright position for 30 to 48 hours at 37 ºC.
- Check the color of the media. Sample is called presumptive positive for *Listeria* species if color of the media changed from yellow/amber color to black/brown color.

13 Interpretation and Test Result Report

Light to dark brown-black colored media at the end of 30 to 48 hour indicates presumptive presence of *Listeria spp*. In order to confirm the negatives, all yellow colored (presumptive negative) tubes have to be incubated for total of 48 hours. It is recommended that at least one negative control be run in each set of analysis. A negative control is an unused sampling device containing one unit of PDX-LIB, incubated alongside the samples.

14 Internal Validation Studies

14.1. Inclusivity –Exclusivity Studies

Serial dilutions from overnight grown pure cultures of 50 different *Listeria* and 30 different non-*Listeria* cultures were made into sterile peptone. One hundred microliters from log –6 dilution (~1.0E+3 CFU/mL) were plated onto TSAIE plates for estimating the number of cells tested. For the inclusivity studies 0.5 mL (estimated cell concentration ranging from 1 to 52 CFU of *Listeria*) of a dilution aliquot was aseptically transferred onto the tip of the sampling sponge. For the exclusivity studies 0.5 mL (estimated cell concentration ranging from 10,000 to 100,000 CFU of non-*Listeria*) of a dilution aliquot was aseptically transferred onto the tip of the sampling sponge. Then sampling device was placed back into the sterile tube and submerged in 20 mL of PDX-LIB media. The results are given in Table 1a and 1 b respectively.

Table 1 a. **Results of Inclusivity Tests for PDX-LIB**

Description and code	Source	Color	Presumptive Result	API
Listeria innocua 33090	Cow brain	Black	+	Li
Listeria welshmeri 43551 (B)	Food/Food Environment	Black	+	Lm
Listeria monocytogenes 19112 (B)	Food/Food Environment	Black	+	Lm
Listeria monocytogenes 3528	Food/Food Environment	Black	+	Lm
Listeria innocua 2242	Food/Food Environment	Black	+	Li
Listeria monocytogenes 3522	Food/Food Environment	Black	+	Lm
Listeria monocytogenes 2421	Food/Food Environment	Black	+	Lm
Listeria monocytogenes 2397	Food/Food Environment	Black	+	Lm
Listeria monocytogenes 4b rt 652 (B)	Food/Food Environment	Black	+	Lm
Listeria monocytogenes 2395	Food/Food Environment	Black	+	Lm
Listeria ivanovii 700402	Food/Food Environment	Black	+	Liv
Listeria monocytogenes 2392	Food/Food Environment	Black	+	Lm
Listeria monocytogenes 2415	Food/Food Environment	Black	+	Lm
Listeria monocytogenes 19118 (B)	Food/Food Environment	Black	+	Lm
Listeria grayi rt721	Food/Food Environment	Black	+	Lg
Listeria monocytogenes 4b 19115	Human	Black	+	Lm
Listeria grayi 700545 Lab	Unspecified	Black	+	Lg
Listeria monocytogenes 1/2 a rt 651 (B)	Food/Food Environment	Black	+	Lm
PDX-Lm47	Food/Food Environment	Black	+	Lm
Listeria monocytogenes 2417	Food/Food Environment	Black	+	Lm
Listeria monocytogenes 1/2 b rt 541 (B)	Food/Food Environment	Black	+	Lm
PDX-Lm48	Food/Food Environment	Black	+	Lm
PDX-Lm44	Food/Food Environment	Black	+	Lm
PDX-Ls4	Unknown	Black	+	Ls
Listeria welshmeri 35967 Lab	Unknown	Black	+	Lw
Listeria monocytogenes 2422	Food/Food Environment	Black	+	Lm
Listeria monocytogenes 2413	Food/Food Environment	Black	+	Lm
U of MN-Vet Med	Unspecified	Black	+	Lm
U of MN-Vet Med	Unspecified	Black	+	Lm
Listeria innocua 2241	Food/Food Environment	Black	+	Li
Listeria monocytogenes 2426	Food/Food Environment	Black	+	Lm
Listeria monocytogenes 2578	Food/Food Environment	Black	+	Lm
PDX-Li10	Food/Food Environment	Black	+	Li
Listeria monocytogenes 2388	Food/Food Environment	Black	+	Lm
Listeria seeligeri 2232	Unspecified	Black	+	Ls
Listeria monocytogenes 2427	Food/Food Environment	Black	+	Lm
Listeria monocytogenes 3742	Food/Food Environment	Black	+	Lm
U of MN-Vet Med	Unspecified	Black	+	Lm
Listeria innocua 51742 Lab	Unknown	Black	+	Li
PDX-Lw3	Unknown	Black	+	Lw
U of MN-Vet Med	Unspecified	Black	+	Lm
U of MN-Vet Med	Unspecified	Black	+	Lm
PDX-Lm46	Food/Food Environment	Black	+	Lm
Listeria monocytogenes 19114	Food	Black	+	Lm
Listeria monocytogenes 19117	Food/Food Environment	Black	+	Lm
PDX-Lm43	Food/Food Environment	Black	+	Lm
PDX-Lm45	Food/Food Environment	Black	+	Lm
Listeria monocytogenes 2389	Food/Food Environment	Black	+	Lm
Listeria monocytogenes 2396	Food/Food Environment	Black	+	Lm
Listeria monocytogenes 2424	Food/Food Environment	Black	+	Lm
Listeria monocytogenes 15313	Food/Food Environment	Black	+	Lm
U of MN-Vet Med	Unspecified	Black	+	Lm
Listeria monocytogenes 1914	Food/Food Environment	Black	+	Lm
Listeria grayi 19120	Chincilla feces	Black	+	Lg
Listeria monocytogenes 2349	Food/Food Environment	Black	+	Lm
U of MN-Vet Med	Unspecified	Black	+	Lm
Listeria innocua 2249	Food/Food Environment	Black	+	Li
Listeria monocytogenes 3550	Food/Food Environment	Black	+	Lm
U of MN-Vet Med	Unspecified	Black	+	Lm
Listeria monocytogenes 2410	Food/Food Environment	Black	+	Lm
PDX-Li 2	Unknown	Black	+	Li
Listeria monocytogenes 2404	Food/Food Environment	Black	+	Lm
Listeria innocua 3757	Food/Food Environment	Black	+	Li
U of MN-Vet Med	Unspecified	Black	+	Lm
Listeria innocua 3181	Food/Food Environment	Black	+	Li
Listeria innocua 3254	Food/Food Environment	Black	+	Li
Listeria ivanovii 49954	Food, France	Black	+	Liv

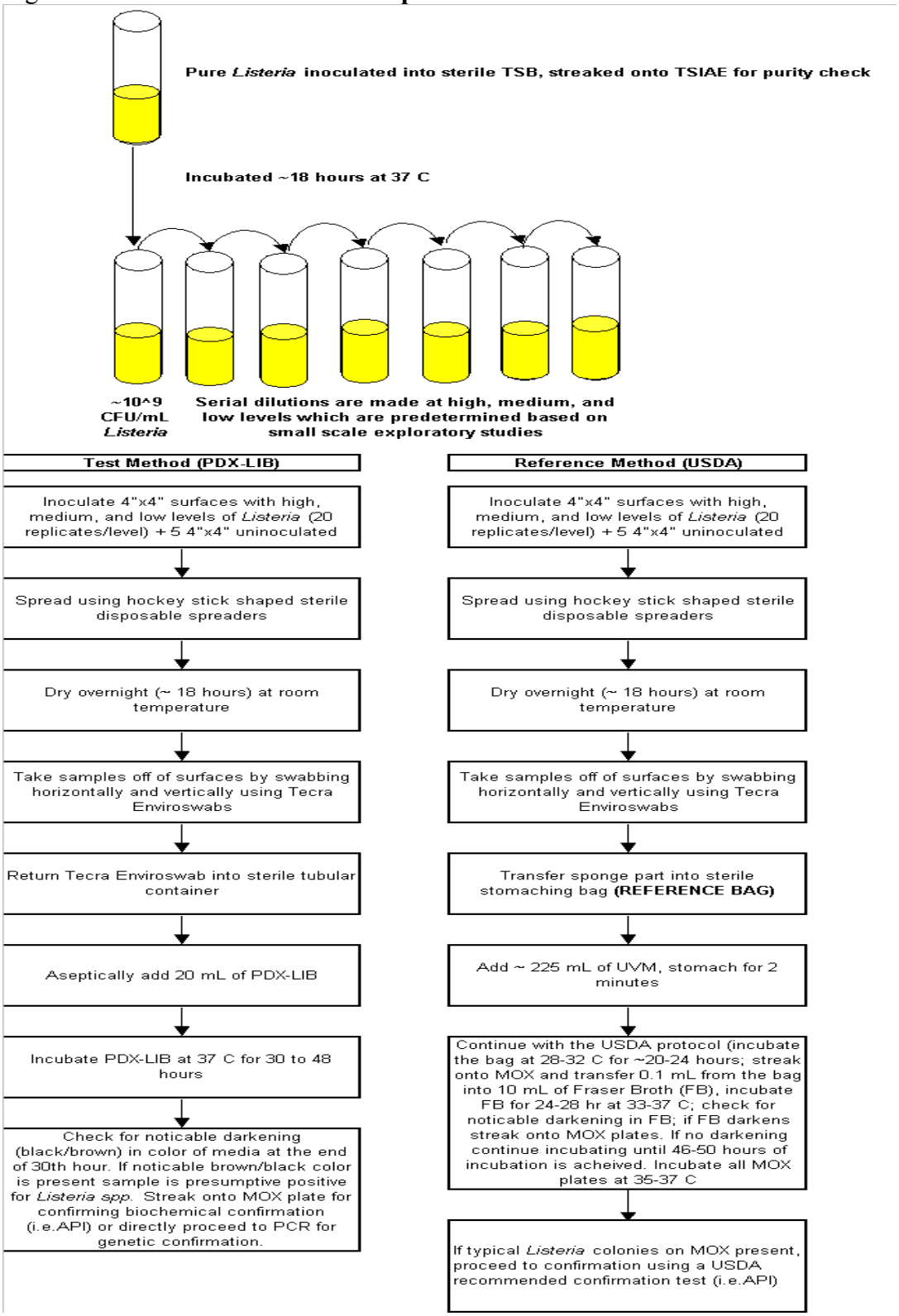
Table 1 b **Results of Exclusivity Tests for PDX-LIB**

Description and code	Source	Color at 30 hours	Color at 48 hours	Presumptive Result	Confirmation
E.coli O157:H7 43895	ATCC-unspecified	Yellow	Yellow	-	Non-Listeria
E. coli 25922	Clinical	Yellow	Yellow	-	Non-Listeria
E.coli 10798	Feces	Yellow	Yellow	-	Non-Listeria
E. coli 51739	ATCC-unspecified	Yellow	Yellow	-	Non-Listeria
Bacillus cereus 11778	ATCC-unspecified	Yellow	Yellow	-	Non-Listeria
Bacillus cereus	Feirtag Culture Collection	Yellow	Yellow	-	Non-Listeria
Bacillus cereus	Feirtag Culture Collection	Yellow	Yellow	-	Non-Listeria
Bacillus stearothermophilus	Feirtag Culture Collection	Yellow	Yellow	-	Non-Listeria
Bacillus stearothermophilus	Feirtag Culture Collection	Yellow	Yellow	-	Non-Listeria
Bacillus subtilis	Feirtag Culture Collection	Yellow	Yellow	-	Non-Listeria
Bacillus circulans	Feirtag Culture Collection	Yellow	Yellow	-	Non-Listeria
Bacillus licheniformis 12759	Cassava tuber	Yellow	Yellow	-	Non-Listeria
Bacillus licheniformis 14580	ATCC-unspecified	Yellow	Yellow	-	Non-Listeria
Entercoccus hire 8043	**ATCC-unspecified**	**Black**	**Black**	+	Non-Listeria
Entercoccus hire 49611	**Pig out**	**Black**	**Black**	+	Non-Listeria
Entercoccus hire 49612	**ATCC-unspecified**	**Black**	**Black**	+	Non-Listeria
Entercoccus hire 35220	**Cattle dung**	**Black**	**Black**	+	Non-Listeria
Enterobacter aerogenes 13048	Sputum	Yellow	Yellow	-	Non-Listeria
Enterobacter cloacae 13047	Spinal fluid	Yellow	Yellow	-	Non-Listeria
Enterococcus feacalis 29212	**Urine**	**Yellow**	**Black**	+	Non-Listeria
Enterococcus feacalis 19433	ATCC-unspecified	Yellow	Yellow	-	Non-Listeria
Enterococcus durans 19432	ATCC-unspecified	Yellow	Yellow	-	Non-Listeria
Enterococcus gallinarum 49573	**Chicken intestine**	**Yellow**	**Black**	+	Non-Listeria
Enterococcus avium 14025	**ATCC-unspecified**	**Black**	**Black**	+	Non-Listeria
Enterococcus casseliflavus 25788	Plants	Yellow	Yellow	-	Non-Listeria
Staphylococcus aureus 35548	ATCC-unspecified	Yellow	Yellow	-	Non-Listeria
Staphylococcus aureus 8095	R-tech labs-unspecified	Yellow	Yellow	-	Non-Listeria
Staphylococcus aureus 25923	Clinical	Yellow	Yellow	-	Non-Listeria
Staphylococcus aureus 51740	Margarine	Yellow	Yellow	-	Non-Listeria
Klebsielle pneumoniae 13883	ATCC-unspecified	Yellow	Yellow	-	Non-Listeria
Klebsielle pneumoniae 27799	Urine	Yellow	Yellow	-	Non-Listeria
Proteius mirebolis 25933	Human vagina	Yellow	Yellow	-	Non-Listeria
Micrococcus luteus 4698	ATCC-unspecified	Yellow	Yellow	-	Non-Listeria
Pseudomonas aerusinosa 27853	Blood culture	Yellow	Yellow	-	Non-Listeria
Staphylococcus epidermidis 1228	R-tech labs-unspecified	Yellow	Yellow	-	Non-Listeria
Proteus vulgaris 8427	Inner ear infection	Yellow	Yellow	-	Non-Listeria
Streptecoccus pyogenes 19615	Pharynx of a child	Yellow	Yellow	-	Non-Listeria

14.2. Method Comparison Studies

Method comparison studies were done for 4 different *Listeria* species paired with four different common food environmental surfaces; *Listeria innocua* (Li) on ceramic tiles, *L.ivanovii* (Liv) on stainless steel, *L. welshmeri* (Lw) on plastic surface, and *L. monocytogenes* (Lm) in presence of one log excess *E.coli* on sealed concrete.

Figure 1. **Flow chart of method comparison studies**



14.2.1. Li on Ceramic Tiles:

Serial logarithmic dilutions of overnight culture of Li in TSB were made into sterile TSB. Aliquots containing 100 µL of log –6 and log -7 dilutions were plated onto MOX and TSAIE plates for estimating the number of cells loaded onto 4"x 4" ceramic tiles.

Ceramic tiles at 4x4 inches in dimensions were bought from a local Home Depot. They were wrapped in aluminum foil and autoclaved for 15 min at 121 ºC. All tiles were kept wrapped at room temperature (RT) until the time of inoculation. The inoculation of surfaces were done at 0.250 mL / surface from the log –4 (high) and log-5 (medium) dilutions, corresponding approximately to 4.67 log CFU and 3.67 log CFU respectively, twenty replicates for each level, per method. Inoculations on surfaces were spread within the corresponding 4"x4" zone with the help of sterile spreaders. Surfaces were left to dry for at least 18 hours at RT.

After the samples were dried on the surfaces for a minimum of 18 hours, they were removed by swabbing using the Tecra Enviroswabs. Both the test method (PDX-LIB) and the reference method (USDA) (10) was done in twenty replicates per level (high and low) of inoculations.

In order to confirm the presence-absence of *Listeria* in all test samples, all tubes of PDX-LIB were streaked onto MOX plates at the end of 48th hour of incubation. Dark gray colonies with black zones grown on MOX were forwarded for microscopy and API confirmation. The raw data for Li on tile is given in Appendix I-A. Summary of the data for Li on tile is given in Table 2.

As seen from the Table 2 below, PDX-LIB Labor Saver was more sensitive than the reference method. The reference method resulted in fewer positives than the test method at both levels. Chi squared value also showed that two methods were significantly different from each other at the "medium level" testing of "Li on ceramic tiles".

14.2.2. Liv on Stainless Steel

Serial logarithmic dilutions of overnight culture of Liv in TSB were made into sterile TSB. A one-hundred microliter volume of log –6 and log -7 dilutions was plated onto MOX and TSAIE plates for estimating the number of cells loaded onto 4"x 4" zones on stainless steel.

Stainless steel sheets were bought from Metal Supermarket Inc, Roseville, MN. They were wrapped in aluminum foil and autoclaved for 15 min at 120 ºC. Stainless steel sheets were kept wrapped until the time of inoculation. The inoculation of surfaces were done at 0.250 mL / surface from the log –1 (high) and log-2 (medium) dilutions, corresponding approximately to 7.07 log CFU and 6.07 log CFU consecutively, twenty replicates for each level per method. Inoculations on surfaces were spread within the corresponding 4"x4" zone with the help of a disposable sterile spreader. The surfaces were left to dry for a minimum of 18 hours at room temperature.

After the samples were dried on the surfaces for a minimum of 18 hours, they were removed by swabbing using Tecra Enviroswabs. Both test method (PDX-LIB-Labor Saver) and reference method (USDA) (10) was done in twenty replicates per level (high and low) of inoculations.

In order to confirm the presence-absence of *Listeria* in all test samples, all tubes of PDX-LIB Labor Saver were streaked onto MOX plates at the end of 48th hour of incubation. Dark gray colonies with black zones grown on MOX were forwarded for microscopy and API confirmation. The raw data for Liv on stainless steel is given in Appendix I-B. The summary of the data for Liv on stainless steel is given in Table 2

As seen from Table 2, PDX-LIB Labor Saver was more sensitive than the reference method. The reference method resulted in far less positive results than the test method at both of the levels tested. Furthermore, chi squared values also suggested that the test method and the reference method were significantly different at both of the levels tested.

14.2.3. Lw on Plastic:

Serial logarithmic dilutions of overnight culture of Lw in TSB were made into sterile TSB. A one-hundred microliter volume of log –6 and log -7 dilutions was plated onto MOX and TSAIE plates for estimating the number of cells loaded onto 4"x 4" zones on plastic.

Polypropylene plastic cutting boards were bought from a local department store. 4"x4" zones were marked on the surface of the cutting boards. They were wrapped in aluminum foil and autoclaved for 15 min at 120 ºC. Plastic cutting

boards were kept wrapped until the time of inoculation. The inoculation of surfaces were done at 0.250 mL / surface from the log –4 (high) and log-6 (low) dilutions, corresponding approximately to 5.18 log CFU and 3.18 log CFU of Lw consecutively, twenty replicates for each level per method. Inoculations on surfaces were spread within the corresponding 4"x4" zone with the help of a disposable sterile spreader. After that surfaces were left to dry min 18 hours at room temperature.

After the samples were dried on the surfaces for a minimum of 18 hours, they were removed by swabbing using Tecra Enviroswabs. Both test method (PDX-LIB-Labor Saver) and reference method (USDA) (10) was done in twenty replicates per level (high and low) of inoculations.

In order to confirm the presence of *Listeria* in all test samples, all samples were streaked onto MOX after at the end of 48th hour at 37 ºC. Dark gray colonies with black zones grown on MOX were forwarded for microscopy and API confirmation. The raw data for Lw on plastic is given in Appendix I-C. The summary of the data for Lw on plastic is given in Table 2.

As seen from Table 2, PDX-LIB Labor Saver was about as sensitive as the reference method. The reference method gave two more positives at the low level than the test method, whereas the test method gave one more true positive result than the reference method at the high level. This difference might be due to the limited ability of the sampling device to pick up the cells from the rough surface of the polypropylene cutting board. Chi squared values also suggested that the test method and the reference method were not significantly different at the low level.

14.2.4. Lm in 10x *E.coli* on sealed concrete:

Lm in 10x *E.coli* stock culture was made by adding 0.1 mL of overnight grown Lm (10^9 CFU/mL) and 1 mL of overnight grown *E.coli* (10^9 CFU/mL) into 9 mL of TSB. Serial logarithmic dilutions of Lm in 10x *E.coli* were made into sterile TSB. A one-hundred microliter volume of log -5, log –6 and log -7 (for E. coli) dilutions were plated onto MOX and TSAIE plates for estimating the number of cells loaded onto 4"x 4" zones on sealed concrete blocks. Number of cells on MOX was used to estimate number of CFU Lm /mL, and number of beige colonies on TSAIE was used to estimate the number of CFU *E.coli* / mL.

Concrete blocks (15.5"x7.5"x3.5"; length x width x depth) were purchased from a local Home Depot. Blocks were sealed with a solvent-based concrete sealer (BW Crete Seal 25 LV, St. Paul, MN). After a minimum of two coats of sealant was placed on the blocks, they were dried in a chemical hood until all the solvent had evaporated (minimum of 24 hours). Zones of 4"x4" were marked on the sealed side using a permanent marker. Before inoculations, sealed concrete blocks were sprayed with ethanol and allowed to air dry. The ethanol was air dried for at least

20 minutes but no more than an hour. The concrete surfaces were not rinsed after the second application of ethanol was allowed to air dry. The inoculation of sealed concrete surfaces were done at 0.250 mL / surface from the log –4 *E.coli* / log-5 Lm (high) and log-5 E.coli / log-6 Lm (low) dilutions, corresponding approximately to 3.85 log CFU and 2.85 log CFU respectively, twenty replicates for each level, per method. Inoculations on surfaces were spread within the corresponding 4"x4" zone. Surfaces were left to dry for at least 18 hours at RT.

After the samples were dried on the surfaces for a minimum of 18 hours, they were removed by swabbing using Tecra Enviroswabs. Both test method (PDX-LIB-Labor Saver) and reference method (USDA) (10) was done in twenty replicates per level (high and low) of inoculations.

As seen from Table 2, although the test method yielded in two false negatives in each level, it performed much better than the reference method. The reference method missed a total of 12 true positives at the high level and 9 true positives at the low level. Chi squared values also suggested that there was a significant difference between the test method and the reference method at both of the levels tested.

1
2 Table 2. Summary of the method comparison studies based on 48 hour results
3
4

				TEST (PDX -LIB Labor Savor)			REFERENCE (USDA)	Chi Squared Value
	Levels	Inoculation (CFU/surface)	Replicates	Presumptive Positives	Confirmed	False Negatives	Confirmed Positives	
Listeria innocua on ceramic tile	- Ctrl	0	5	0	0	0	0	NA
	Medium	4669	20	19	19	0	6	15.36
	High	46688	20	20	20	0	19	0
Listeria ivanovii on stainless steel	- Ctrl	0	5	0	0	0	0	NA
	Medium	1175000	20	20	20	0	5	20.91
	High	11750000	20	19	19	0	7	13.3
Listeria welshmeri on plastic	- Ctrl	0	5	0	0	0	0	NA
	Low	1506	20	15	15	0	17	1.41
	High	150563	20	20	20	0	19	0
Listeria monocytogenes in 10x E.coli on sealed concrete	- Ctrl	0	5	0	0	0	0	NA
	Medium	715	20	8	9	1	0	7.66
	High	7156	20	14	15	1	3	10.23

14.3. Ruggedness Studies

Ruggedness parameters studied were incubation times (28 hours, 32 hours and 46 and 50 hours) and incubation temperatures (36 and 38 °C). Two positive controls (one *L.monocytogenes* and one *L.innocua*) and one negative control (*E.coli*) were tested in 5 replicates at the 1.0E+3 CFU/mL for *Listeria spp* and at 1.0E+7 CFU/mL for *E.coli*. These tests were done on different days as recommended. The summary of the results is given in Table 3.

Table 3. **Ruggedness Studies for PDX-LIB**

		Variable Incubation hours at fixed incubation temperature (37 °C)						Vairable incubation temperatures at fixed incubation times (30 hours and 48 hours)	
	Replicates	28 hour	30 hour	32 hour	46hour	48hour	50 hour	36°C*	38°C*
Listeria innocua	A	+	+	+	+	+	+	++	++
	B	+	+	+	+	+	+	++	++
	C	+	+	+	+	+	+	++	++
	D	+	+	+	+	+	+	++	++
	E	+	+	+	+	+	+	++	++
Listeria monocytogenes	A	+	+	+	+	+	+	++	++
	B	+	+	+	+	+	+	++	++
	C	+	+	+	+	+	+	++	++
	D	+	+	+	+	+	+	++	++
	E	+	+	+	+	+	+	++	++
E.coli	A	-	-	-	-	-	-	--	--
	B	-	-	-	-	-	-	--	--
	C	-	-	-	-	-	-	--	--
	D	-	-	-	-	-	-	--	--
	E	-	-	-	-	-	-	--	--
	Neg	-	-	-	-	-	-	-	-

*** Two signs in the each cell represents 30 hour and 48 hour color change profiles;**
+: Black , - : Yellow

14.4. Lot to lot variability and shelf stability

Lot to lot variability studies were done in 5 replicates per level of each microorganism for each of the three production lots tested (Table 4). Shelf life studies were done using three indicator microorganisms in triplicates (Table 5). *L. monocytogenes* ATCC 19119, *L.grayi* (*Listeria grayi 700545 Lab*) and *E. faecalis* ATCC 19433. Five hundred microliters of culture dilutions were inoculated onto the tip of Tecra EnviroSwab, which was then fully submerged into 20 mL of PDX-LIB in its original sterile container.

Table 4: Lot to lot variability studies

		Lot 50175		Lot 50191		Lot 50200	
	Sample	30hr	48hr	30hr	48hr	30hr	48hr
Listeria innocua	Li A	+	+	+	+	+	+
	B	+	+	+	+	+	+
	C	+	+	+	+	+	+
	D	+	+	+	+	+	+
	E	+	+	+	+	+	+
Listeria monocytogenes	Lm A	+	+	+	+	+	+
	B	+	+	+	+	+	+
	C	+	+	+	+	+	+
	D	+	+	+	+	+	+
	E	+	+	+	+	+	+
E.coli	A	-	-	-	-	-	-
	B	-	-	-	-	-	-
	C	-	-	-	-	-	-
	D	-	-	-	-	-	-
	E	-	-	-	-	-	-
	Neg	-	-	-	-	-	-

+: Black , - : Yellow

Table 5. Shelf life studies of PDX-LIB

		Lot 63 at 9 months of refrigerated storage (new QC)		
	~ CFU	A	B	C
Listeria monocytogenes	10	+	+	+
	100	+	+	+
Listeria grayi	190	+	+	+
	1900	+	+	+
Enterococcus fecealis	38000	-	-	-
	380000	+	-	-

+: Black , - : Yellow

15 Discussion

PDX-LIB Labor Saver is an easy to use and interpret screening test for *Listeria spp* in environmental samples. Inclusivity and exclusivity studies revealed that PDX-LIB Labor Saver is very comprehensive for the detection of *Listeria* species at very low levels (1-52 cells). In regard to specificity, high levels of *Enterococcus* species remain to be a top group for potential false positive results. False positive results, although not desired by the typical end user, still tells a great deal about the overall microbial cleanliness of the areas sampled. Learning about the presence of *Enterococcus* species is important for another aspect, some *Enterococcus* species, namely *E. hirae* produces bacteriocin inhibitory to *Listeria* species (11). Other methods might miss the true presence of *Listeria* if the sample contains *Enterococcus* species.

PDX-LIB Labor Saver was found to be at least as sensitive as the reference method. In fact PDX-LIB Labor Saver has outperformed the reference method in most of the method comparison studies.

Regarding the ruggedness studies, recommended parameters have been studied for PDX-LIB. Results of the ruggedness studies suggested selected deviations from test parameters did not interfere with the true detection of microorganisms selected.

Lot-to-lot variability studies showed that there was no difference between the production lots. Shelf life studies revealed that PDX-LIB is shelf-stable at 10 months of refrigerated storage.

PDX-LIB Labor Saver is a unique, easy to perform rapid detection test for *Listeria* species in environmental samples. Since PDX-LIB Labor Saver is a self-contained test, it minimizes cross contamination. Furthermore PDX-LIB labor saver is compatible with PCR based confirmations (data not shown). This compatibility provides a more economical solution to the *Listeria* screening without any compromise in sensitivity. It is expected that PDX-LIB Labor Saver will revolutionize *Listeria* monitoring in the environmental samples.

Conclusion

Significant improvement has been made in the PDX-LIB method by simplifying the test protocol. The performance of PDX-LIB Labor Saver has been tested rigorously in AOAC-guideline studies. The results suggested that PDX-LIB Labor Saver was associated with desirable levels of performance characteristics when compared to USDA methods while providing presumptive results in a single stage test.

16 References

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11. Siragusa, G.R. (1992) Production of Bacteriocin Inhibitory to *Listeria* species by *Enterococcus hirae*. Applied and Environmental Microbiology Volume 58, No. 11, pages 3508-3513.

17 Attachments

17.1 Package Inserts: Users Guide and Instruction for Use (IFU) Sheets

Paradigm Diagnostics *Listeria* Indicator Broth (PDX-LIB)
User Guide
Listeria species Test Kit
Catalog Number:

25003-25
25004-100
25005-100
25009-50

Intended Use

Paradigm Diagnostics *Listeria* indicator broth **(PDX-LIB)** is intended to be used in the food processing environment and on food contact surfaces to detect the presence of *Listeria* species. A color change from yellow to light brown/black is considered presumptive positive. Applicability of PDX-LIB is limited for selected common *Listeria spp* (*Listeria monocytogenes*, *L. innocua*, *L. ivanovii*, and *L.welshmeri*) on selected common surface types (Sealed concrete, ceramic tile, stainless steel, and plastic). **AOAC-RI PTM validation studies were conducted at 4 inch x 4-inch surface areas.**

Scientific Principle of the Test

PDX-LIB contains a patented formula of antibiotics, growth enhancers and color changing compounds. The antibiotics function synergistically to inhibit most non-*Listeria* microorganisms. Growth enhancers provide recovery nutrients to support the growth of sub-lethally injured *Listeria*. Indicator compounds will turn the broth from yellow to black by utilizing the β-glucosidase enzyme produced by *Listeria* species. A brown to black color 30-48 hours at 37^{o}C indicates a presumptive positive test for *Listeria* spp. **Positive results can be read as early as 30 hours. Results cannot be considered negative until samples have been incubated for 48 hours.**

Additional Notes

Paradigm Diagnostics recommends the use of TECRA EnviroSwab (BioTrace International Bioproducts, Bothell, Washington, 425-398-7993) as sampling device for increased reliability of **PDX-LIB**.

Materials and Equipment Required

Tecra Enviroswabs for sampling and an incubator capable of maintaining 37ºC ± 1ºC (e.g. heat block, water bath, air incubator).

Confirmation Step

Presumptive positive samples from food contact surfaces or the food processing environment can be confirmed streaking presumptive positive samples onto commonly used selective *Listeria* agar plates such as Modified Oxford Agar or Palcam agars. Typical *Listeria* colonies (dark gray colonies with black zones, generally with dimples) on MOX are used in confirmation protocols given in FDA/BAM (1) or USDA (2) methods.

Disposal

Decontaminate the **PDX-LIB** after use by autoclave, bleach or other disinfectants in accordance with local, state and federal regulations.

Product Shelf Life

PDX-LIB is stable **for 9 months** at refrigeration temperatures. The expiration date appears on the label along with the lot number. Keep PDX-LIB away from light during storage.

Precautions

1. *Listeria monocytogenes* is a dangerous human pathogen. When handling samples that possibly contain *L.monocytogenes*, extreme care should be taken to contain the samples and the enriched samples (presumptive positive tubes). Immuno-compromised individuals and pregnant women are particularly endangered by exposure to *L.monocytogenes* and should not be allowed in the vicinity of the testing.
2. WARNING: Some *Enterococci*, particularly *E.hirea* , *E. avium, E.feacalis*, and *E. gallinarum* could be resulting in presumptive false positive results. However, all media that noticeably darkens in color must be streaked onto MOX. Because, *Enteroccoci* do not grow on MOX plates, false positive result remains at a presumptive level.
3. Use of PDX-LIB with some yellow-pigmented sponges should be avoided because of the likely enzyme activity suppression and the resulting possibility of false negative test results.
4. Do not use PDX-LIB past the expiration date that appears on the label
5. Follow standard Good Microbiological Practices where appropriate.

(1) http://www.cfsan.fda.gov/~ebam/bam-10.html
(2)http://www.fsis.usda.gov/Ophs/Microlab/Mlg_8_04.pdf

Warranties and Liabilities

Paradigm Diagnostics Inc warrants the Products manufactured by it will be free from defects in materials and workmanship when used in accordance with the applicable instructions until the expiration date noted on the product packaging. Application protocols suggested by Paradigm are intended to be guidelines to the Buyers of the Products. Each Buyer is expected to validate the applicability of each application protocol to their individual applications. **PARADIGM DIAGNOSTICS MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.**

Paradigm Diagnostics' sole obligation with respect to the foregoing warranties shall be, at its option, to either replace or to refund the purchase price of the Product(s) or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notified Paradigm Diagnostics promptly of any such defect. **PARADIGM DIAGNOSTICS SHALL NOT BE LIABLE FOR ANY DIRECT, INDIRECT OR CONSEQUENTIAL DAMAGES RESULTING FROM ECONOMIC LOSS OR PROPERTY DAMAGES SUSTAINED BY BUYER OR ANY CUSTOMER FROM THE USE OF THE PRODUCT(S).**

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Instructions for Use: PDX-LIB Labor Saver for Environmental Samples

1. SAMPLING STEP: Take an environmental sample following USDA guidelines.

- Remove recommended sponge out of its sterile wrapping.
- Sponge the 4"x 4" area to be tested.
- Return the sponge into the original sampling container.
- Aseptically add one unit (20 mL) of PDX-LIB on top of sponge in sampling container, and snap close the sampling container.
- Incubate upright (sponge fully submerged in PDX-LIB) at 37°C for 30 to 48 hours.

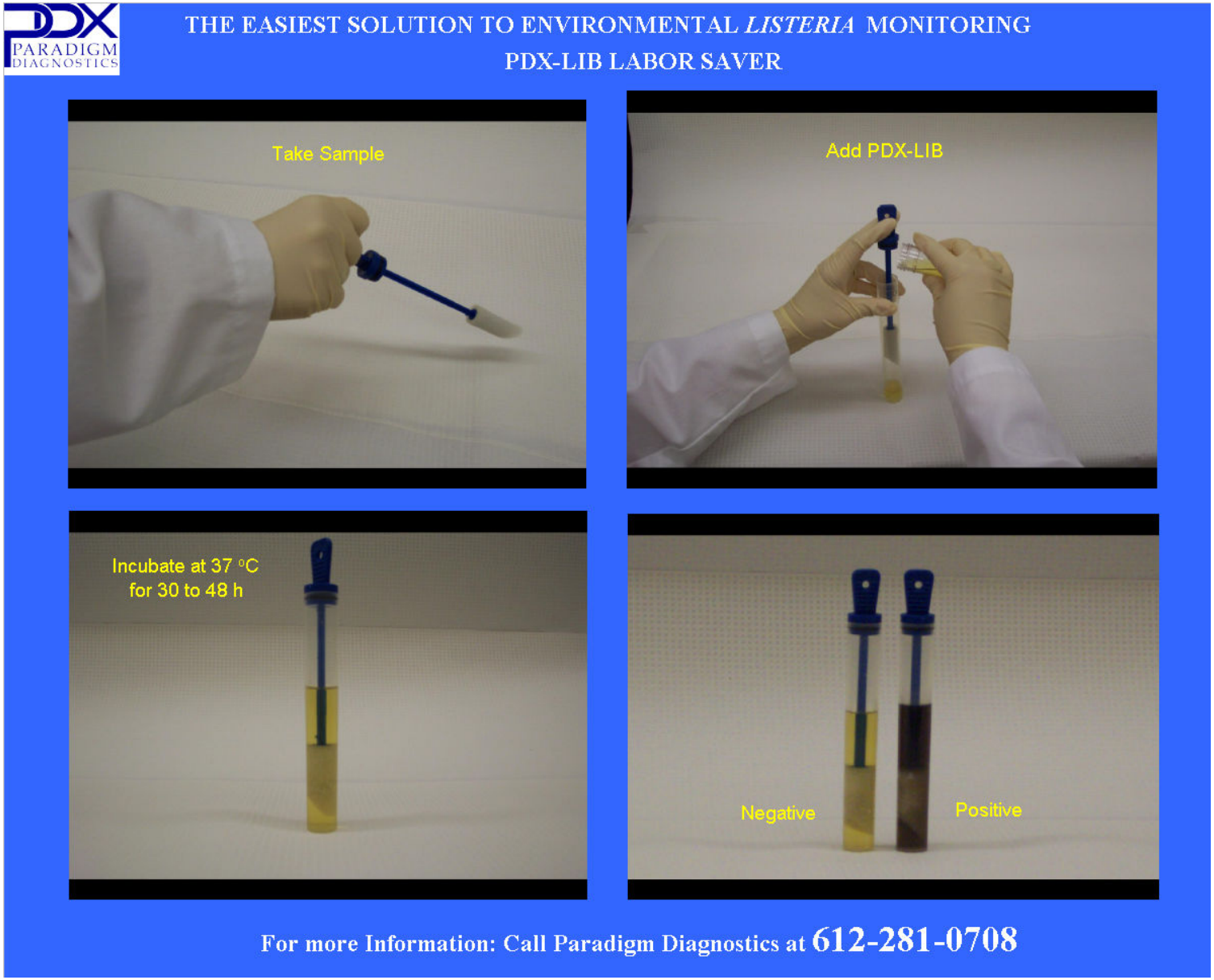
2. **INTERPRETATION STEP:** If color of the media changes from yellow/amber to brown/black, after **30 to 48** hours of incubation at 37°C, the sample is considered presumptive positive for *Listeria spp*. **Positive results can be read as early as 30 hours. Results cannot be considered negative until samples have been incubated for 48 hours. (Note: Although PDX-LIB has been designed to provide results within 30 hours, 48 hours of incubation at 37 °C has been shown to significantly improve the sensitivity of the test, and therefore highly recommended for maximum sensitivity).** As with all experimental protocols, use of negative control (an unused sampling device containing one unit of **PDX-LIB**, incubated along side with the environmental samples) in each set of samples is recommended.

NOTE: For audio-visual instruction, please visit us at

http://www.pdx-inc.com/training/index.html

and click on the link below

Training Video - **PDX-LIB-List 'Labor Saver' Method**


PDX
PARADIGM DIAGNOSTICS
THE EASIEST SOLUTION TO ENVIRONMENTAL *LISTERIA* MONITORING
PDX-LIB LABOR SAVER
Take Sample
Add PDX-LIB
Incubate at 37 °C
for 30 to 48 h
Negative
Positive
For more Information: Call Paradigm Diagnostics at 612-281-0708

APPENDIXES

Appendix I. Method Comparison Data A) Li on Tile

Medium	PDX-LIB				USDA			
	30 hour	48 hour	MOX	API	24 hour	FB	FB MOX	API
A	+	+	+	*Listeria innocua*	+	+		*Listeria innocua*
B	-	+	+	*Listeria innocua*	+	+		
C	-	-	-		-	+	+	*Listeria innocua*
D	+	+	+	*Listeria innocua*	+	+		
E	+	+	+	*Listeria innocua*	-	-		
F	-	+	+	*Listeria innocua*	-	-		
G	-	+	+	*Listeria innocua*	-	-		
H	-	+	+	*Listeria innocua*	-	-		
I	+	+	+	*Listeria innocua*	-	-		
J	+	+	+	*Listeria innocua*	-	-		
K	-	+	+	*Listeria innocua*	-	+	+	*Listeria innocua*
L	+	+	+	*Listeria innocua*	-	-		
M	+	+	+	*Listeria innocua*	-	+	+	*Listeria innocua*
N	+	+	+	*Listeria innocua*	-	-		
O	+	+	+	*Listeria innocua*	-	-		
P	+	+	+	*Listeria innocua*	-	+	+	*Listeria innocua*
Q	+	+	+	*Listeria innocua*	-	-		
R	-	+	+	*Listeria innocua*	-	-		
S	+	+	+	*Listeria innocua*	+	+		*Listeria innocua*
T	-	+	+	*Listeria innocua*	-	-		

High	PDX-LIB				USDA			
Sample	30 hour	48 hour	MOX	API	24 hour	FB	FB MOX	API
A	+	+	+	*Listeria innocua*	+	+		*Listeria innocua*
B	+	+	+	*Listeria innocua*	+	+		*Listeria innocua*
C	+	+	+	*Listeria innocua*	+	+		*Listeria innocua*
D	+	+	+	*Listeria innocua*	-	+	+	*Listeria innocua*
E	+	+	+	*Listeria innocua*	-	+	+	*Listeria innocua*
F	+	+	+	*Listeria innocua*	+	+		*Listeria innocua*
G	+	+	+	*Listeria innocua*	-	-		
H	+	+	+	*Listeria innocua*	+	+		*Listeria innocua*
I	+	+	+	*Listeria innocua*	+	+		*Listeria innocua*
J	+	+	+	*Listeria innocua*	+	+		*Listeria innocua*
K	+	+	+	*Listeria innocua*	+	+		*Listeria innocua*
L	+	+	+	*Listeria innocua*	+	+		*Listeria innocua*
M	+	+	+	*Listeria innocua*	+	+		*Listeria innocua*
N	+	+	+	*Listeria innocua*	+	+		*Listeria innocua*
O	+	+	+	*Listeria innocua*	+	+		*Listeria innocua*
P	+	+	+	*Listeria innocua*	+	+		*Listeria innocua*
Q	+	+	+	*Listeria innocua*	+	+		*Listeria innocua*
R	+	+	+	*Listeria innocua*	+	+	+	*Listeria innocua*
S	+	+	+	*Listeria innocua*	-	+		*Listeria innocua*
T	+	+	+	*Listeria innocua*	+	+		*Listeria innocua*
Blk 1	-	-	-		-	-		
Blk 2	-	-	-		-	-		
Blk 3	-	-	-		-	-		
Blk 4	-	-	-		-	-		
Blk 5	-	-	-		-	-		

24 hour=Presence of *Listeria* on MOX from 24 hour UVM; FB: Darkening of Fraser Broth; FB MOX: presence of *Listeria* colonies on MOX from darkened FB.

Appendix I. Method Comparison Data B) Liv on stainless steel

Medium	PDX-LIB				USDA			
	30 hour	48 hour	MOX	API	24 hour	FB	FB MOX	API
A	-	+	+	*Listeria ivanovii*	-	+	+	*Listeria ivanovii*
B	-	+	+	*Listeria ivanovii*	-	-		
C	-	+	+	*Listeria ivanovii*	-	-		
D	-	+	+	*Listeria ivanovii*	-	-		
E	-	+	+	*Listeria ivanovii*	-	+	+	*Listeria ivanovii*
F	-	+	+	*Listeria ivanovii*	-	-		
G	-	+	+	*Listeria ivanovii*	-	-		
H	-	+	+	*Listeria ivanovii*	-	-		
I	-	+	+	*Listeria ivanovii*	-	+	+	*Listeria ivanovii*
J	-	+	+	*Listeria ivanovii*	-	-		
K	-	+	+	*Listeria ivanovii*	-	-		
L	-	+	+	*Listeria ivanovii*	-	-		
M	-	+	+	*Listeria ivanovii*	-	-		
N	-	+	+	*Listeria ivanovii*	-	-		
O	-	+	+	*Listeria ivanovii*	-	+	+	*Listeria ivanovii*
P	-	+	+	*Listeria ivanovii*	-	-		
Q	-	+	+	*Listeria ivanovii*	-	-		
R	-	+	+	*Listeria ivanovii*	-	+	+	*Listeria ivanovii*
S	-	+	+	*Listeria ivanovii*	-	-		
T	-	+	+	*Listeria ivanovii*	-	-		
High	PDX-LIB				USDA			
Sample	30 hour	48 hour	MOX	API	24 hour	FB	FB MOX	API
A	-	+	+	*Listeria ivanovii*	-	-		
B	-	+	+	*Listeria ivanovii*	-	-		
C	-	-	-		-	-		
D	-	+	+	*Listeria ivanovii*	-	-		
E	-	+	+	*Listeria ivanovii*	-	-		
F	+	+	+	*Listeria ivanovii*	-	-		
G	-	+	+	*Listeria ivanovii*	-	-		
H	-	+	+	*Listeria ivanovii*	-	-		
I	-	+	+	*Listeria ivanovii*	-	+	+	*Listeria ivanovii*
J	-	+	+	*Listeria ivanovii*	-	+	+	*Listeria ivanovii*
K	+	+	+	*Listeria ivanovii*	-	-		
L	+	+	+	*Listeria ivanovii*	-	+	+	*Listeria ivanovii*
M	-	+	+	*Listeria ivanovii*	-	+	+	*Listeria ivanovii*
N	-	+	+	*Listeria ivanovii*	-	-		
O	-	+	+	*Listeria ivanovii*	-	-		
P	+	+	+	*Listeria ivanovii*	-	+	+	*Listeria ivanovii*
Q	-	+	+	*Listeria ivanovii*	-	+	+	*Listeria ivanovii*
R	+	+	+	*Listeria ivanovii*	-	-		
S	-	+	+	*Listeria ivanovii*	-	+	+	*Listeria ivanovii*
T	-	+	+	*Listeria ivanovii*	-	-		
Blk 1	-	-	-		-	-		
Blk 2	-	-	-		-	-		
Blk 3	-	-	-		-	-		
Blk 4	-	-	-		-	-		
Blk 5	-	-	-		-	-		

24 hour=Presence of *Listeria* on MOX from 24 hour UVM; FB: Darkening of Fraser Broth; FB MOX: presence of *Listeria* colonies on MOX from darkened FB.

Appendix I. Method Comparison Data C) Lw on plastic

Low	PDX-LIB				USDA			
	30 hour	48 hour	MOX	API	24 hour	FB	FB MOX	API
A	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
B	-	+	+	*Listeria welshmeri*	-	+	+	*Listeria welshmeri*
C	-	+	+	*Listeria welshmeri*	-	+	+	*Listeria welshmeri*
D	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
E	-	+	+	*Listeria welshmeri*	-	+	+	*Listeria welshmeri*
F	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
G	-	+	+	*Listeria welshmeri*	-	-		
H	-	-	-		-	+		*Listeria welshmeri*
I	-	-	-		+	+		*Listeria welshmeri*
J	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
K	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
L	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
M	-	+	+	*Listeria welshmeri*	-	-		
N	-	+	+	*Listeria welshmeri*	-	-		
O	-	-	-		+	+		*Listeria welshmeri*
P	-	-	-		+	+		*Listeria welshmeri*
Q	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
R	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
S	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
T	-	-	-		+	+		*Listeria welshmeri*
High	PDX-LIB				USDA			
Sample	30 hour	48 hour	MOX	API	24 hour	FB	FB MOX	API
A	+	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
B	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
C	+	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
D	+	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
E	+	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
F	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
G	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
H	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
I	+	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
J	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
K	-	+	+	*Listeria welshmeri*	-	-		
L	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
M	+	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
N	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
O	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
P	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
Q	+	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
R	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
S	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
T	-	+	+	*Listeria welshmeri*	+	+		*Listeria welshmeri*
Blk 1	-	-	-		-	-		
Blk 2	-	-	-		-	-		
Blk 3	-	-	-		-	-		
Blk 4	-	-	-		-	-		
Blk 5	-	-	-		-	-		

24 hour=Presence of *Listeria* on MOX from 24 hour UVM; FB: Darkening of Fraser Broth; FB MOX: presence of *Listeria* colonies on MOX from darkened FB.

Appendix I. Method Comparison Data D) Lm in 10x *E.coli* on sealed concrete

Medium	PDX-LIB				USDA			
	30 hour	48 hour	MOX	API	24 hour	FB	FB MOX	API
A	-	-	-		-	-		
B	-	+	+	*Listeria monocytogenes*	-	-		
C	-	+	+	*Listeria monocytogenes*	-	-		
D	-	-	-		-	-		
E	-	-	-		-	-		
F	-	-	-		-	-		
G	-	+	+	*Listeria monocytogenes*	-	-		
H	-	-	-		-	-		
I	-	-	-		-	-		
J	-	+	+	*Listeria monocytogenes*	-	-		
K	-	+	+	*Listeria monocytogenes*	-	-		
L	-	+	+	*Listeria monocytogenes*	-	-		
M	-	-	-		-	-		
N	-	-	-		-	-		
O	-	+	+	*Listeria monocytogenes*	-	-		
P	-	-	-		-	-		
Q	+	+	+	*Listeria monocytogenes*	-	-		
R	-	-	-		-	-		
S	-	-	+	*Listeria monocytogenes*	-	-		
T	-	-	-		-	-		
High	PDX-LIB				USDA			
Sample	30 hour	48 hour	MOX	API	24 hour	FB	FB MOX	API
A	-	-	-		-	-		
B	+	+	+	*Listeria monocytogenes*	-	-		
C	-	+	+	*Listeria monocytogenes*	-	-		
D	+	+	+	*Listeria monocytogenes*	-	-		
E	-	+	+	*Listeria monocytogenes*	-	-		
F	+	+	+	*Listeria monocytogenes*	-	-		
G	-	+	+	*Listeria monocytogenes*	+	-		*Listeria monocytogenes*
H	-	+	+	*Listeria monocytogenes*	-	-		
I	-	+	+	*Listeria monocytogenes*	-	-		
J	-	-	-		-	-		
K	-	+	+	*Listeria monocytogenes*	-	+	+	*Listeria monocytogenes*
L	-	+	+	*Listeria monocytogenes*	-	-		
M	-	+	+	*Listeria monocytogenes*	-	-		
N	-	+	+	*Listeria monocytogenes*	+	+		*Listeria monocytogenes*
O	-	-	-		-	-		
P	-	-	-		-	-		
Q	-	+	+	*Listeria monocytogenes*	-	-		
R	-	+	+	*Listeria monocytogenes*	-	-		
S	-	-	-		-	-		
T	-	-	+	*Listeria monocytogenes*	-	-		
Blk 1	-	-	-		-	-		
Blk 2	-	-	-		-	-		
Blk 3	-	-	-		-	-		
Blk 4	-	-	-		-	-		
Blk 5	-	-	-		-	-		

24 hour=Presence of *Listeria* on MOX from 24 hour UVM; FB: Darkening of Fraser Broth; FB MOX: presence of *Listeria* colonies on MOX from darkened FB.