PathogenDx


# Novel Multiple DNA Microarray Assay for Species Discrimination of *Listeria spp* and *Listeria monocytogenes* on Environmental Surfaces

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# Presentation Outline

**Background on Micro and Molecular Biology Diagnostics**

- Introduction to current microbiology and current molecular tests
  - Focus on current *Listeria spp* and *Listeria monocytogenes* diagnostics

**Background on PathogenDx**

- Overview of DNA Microarray Technology – Design and Implementation
  - What is our approach to molecular diagnostic technology and how is that technology designed to solve the technological challenges within the food, agricultural, and medical industry

**PathogenDx Enviro$^{x}$ Food Safety Assay – AOAC Study**

- Background on study design
- Description of the study results

**Conclusion**

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# HISTORICAL PERSPECTIVE ON MICROBIOLOGY PATHOGEN DIAGNOSTICS

**Traditional Microbiologic Pathogen Diagnostics:**

- Plate Based Culture has been the gold standard for identifying and quantitating microorganisms for the past 130 year.
- There has been significant advances in enrichment methods, media types, and incubation conditions.
- Despite these advances, you can only identify what you can culture (less than 1% of known organisms)
- Problem: The limitations in culture-based methods have driven the microbial testing regulations in multiple industries.
    - Expertise, time to answer, sensitivity, specificity, and broad diagnostic content.

**Molecular Based Pathogen Diagnostics:**

- New and advanced methods of molecular detection are changing the way that we practice clinical microbiology, food, agricultural, environmental and cannabis safety.
- Solution: These molecular techniques offer increase sensitivity, specificity and faster turnaround times.

**Gary W. Proop. Molecular Diagnostics for Detection and Characterization of Microbial Pathogens. *Clinical Infectious Diseases*. 2007.**
**Vouga and Greub. Emerging Bacterial Pathogens: The Past and Beyond. *Clinical Microbiology and Infection*. 2015.**
**Lagier et al. Current and Past Strategies for Bacterial Culture in Clinical Microbiology. *Clinical Microbiology Reviews*. 2015.**

# Microbial Diagnostics: An Overview

| Technique | Application Examples | Advantages | Disadvantages |
|---|---|---|---|
| **Cell Based Techniques** | Culture In/On Broad and Selective Media | • Sensitive<br>• Time Tested (Reliable)<br>• Qualitative and Quantitative | • Low specificity<br>• Time and labor intensive<br>• High cost<br>• High expertise required |
| **Immunological** | ELISA | • High-Throughput<br>• Qualitative and Quantitative<br>• Ease of Use | • Detection limits for organism/ antigen with low abundance<br>• Specificity of antibodies |
| **Nucleic Acid Based** | PCR/qPCR | • Rapid and Easy to Perform<br>• Qualitative and Quantitative<br>• Low input amount<br>• Identify non-culturable organisms<br>• High-throughput | • Limited multiplexing capability<br>• Requires enrichment<br>• Requires sample purification<br>• High cost |
| | Sequencing | | • High-cost (time, labor and expertise)<br>• Need for Specific Primers<br>• Poor limit of detection<br>• Requires enrichment |
| | Hybridization (Southern or Northern Blot ex.) | | • Limited multiplexing<br>• Requires enrichment |
| | DNA/RNA Microarray | | • Medium expense<br>• Upfront investment in training |

# LISTERIA SPP AND LISTERIA MONOCYTOGENES DIAGNOSTIC ASSAYS

## *Listeria* Background

- *Listeria* is a Gram-positive bacterium commonly found in the soil and on surfaces of food processing and distribution facilities.
  - There are six species: *L monocytogenes, L innocua, L ivanovii, L seeligeri, L welshimeri,* and *L grayi*
  - Two species, *L monocytogenes* and *L ivanovii*, are recognized as pathogenic but there are reports of additional organisms causing illness
- *L. monocytogenes* is a foodborne intracellular pathogen whereby eating contaminated food can lead to listeriosis, with death rates at 20-30%.
- The majority of diagnostic assay, both traditional and molecular do not have the capacity to speciate *Listeria*
- Speciation as well as detection will greatly improve our understanding of *Listeria* pathogenicity and contamination rates

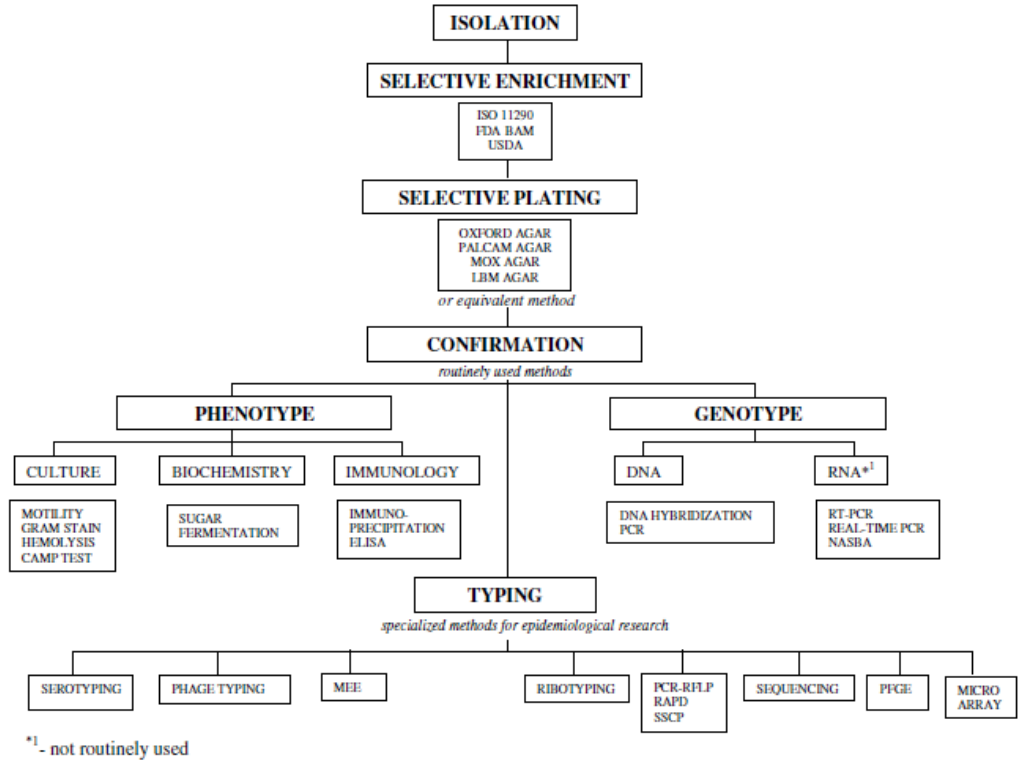


Fig. 1. Overview of isolation, identification and typing methods for Listeria and L. monocytogenes in foods and environmental samples.

**Gasanov et al. Methods for the isolation and identification of *Listeria* spp and *Listeria monocytogenes*: a review. FEMS Microbiology Reviews 29 (2005) 851-875.**
**Liu et al. A minireview of the methods for *Listeria monocytogenes* detection. Food Anal. Methods. 3 July 2017.**

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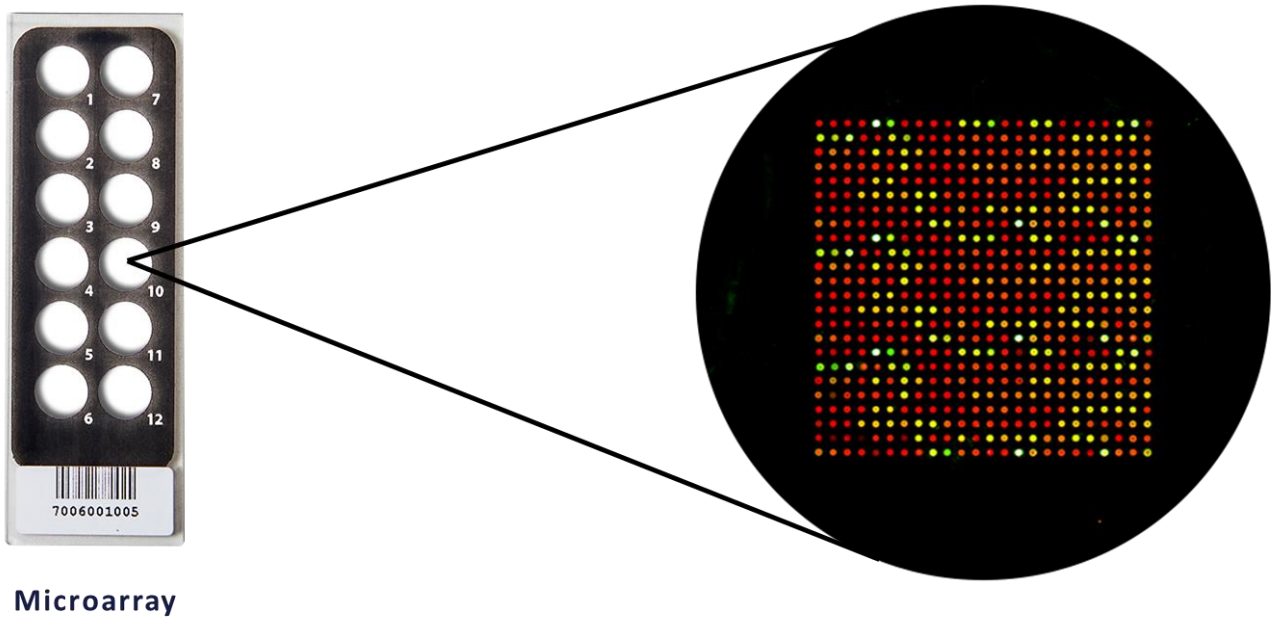
# PATHOGENDX TECHNOLOGY ARCHITECTURE

DNA microarray technology is a high-throughput diagnostic tool for the detection and identification of pathogenic organisms. The development of DNA microarrays is divided into two sections:

1. **In-silico Design:** Sequence alignment, primer and probe design
2. **In-vitro Testing:** DNA extraction, PCR primer and probe concentration and amplification, and DNA hybridization probe specificity

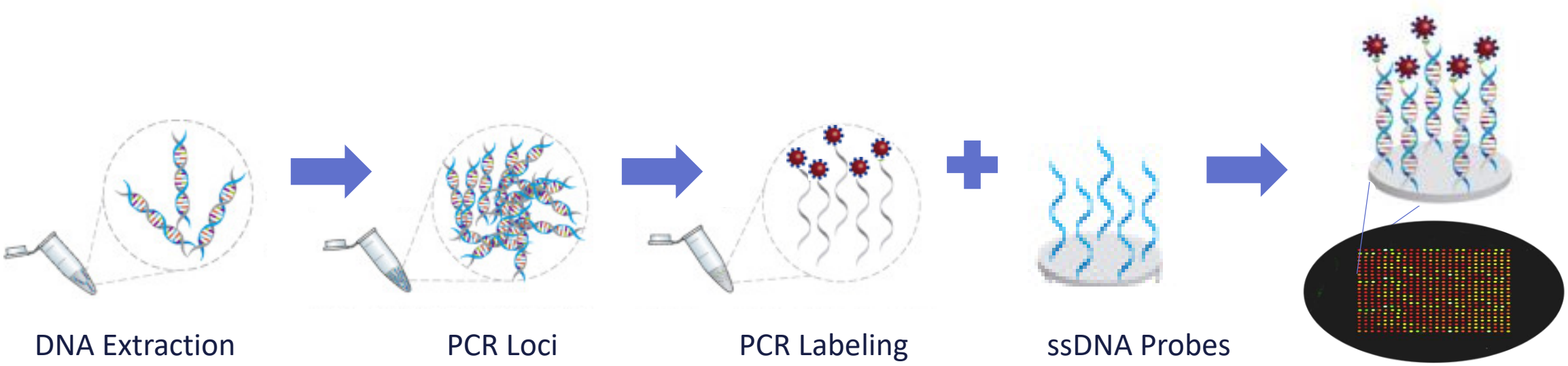



Microarray

*Flexible Design, Adaptable, High-Throughput, Time and Cost Effective, Sensitive and Specific*

# DNA/RNA MICROARRAY PROCESS DESCRIPTION

DNA microarray technology is a high-throughput diagnostic tool for the detection and identification of pathogenic organisms.



DNA Extraction | PCR Loci | PCR Labeling | ssDNA Probes | DNA Hybridization

*Flexible Design, High-Throughput, Time and Cost Effective, Sensitive and Specific, without Sample Enrichment*

# Enviro[x] Array Content

## Enviro[x]

Simple.
Cost Effective.
Comprehensive.


**TARGET ORGANISMS:** BACTERIAL

- Pan Bacterial (TAB)
- Bile-Tolerant Gram-neg
- Enterobacteriaceae
- *Salmonella/ Enterobacter*
- ***Salmonella spp***
- *Escherichia/Shigella*
- *Escherichia* stx1
- *Escherichia* stx2
- *Escherichia eae*
- *Pseudomonas spp*
- ***Listeria spp***
- ***Listeria monocytogenes***
- *Campylobacter spp*
- *Xanthamonas*
- *Aeromonas spp*
- *Bacillus spp*
- *Vibrio spp*
- *Staphylococcus spp*
- *Hafnia*
- *Klebsiella*
- *Serratia*
- *Klebsiella*
- *Chromobacterium spp*
- *Bacillus spp*
- *Streptomyces spp*
- *Legionella*
- *Alkanindiges*
- *Citrobacter*
- *Clostridium spp*
- *Yersinia*
- *Panteoa*

**TARGET ORGANISMS***:* FUNGAL

- Pan Fungal (TY&M)
- *Aspergillus spp*
- *Botrytis spp*
- *Penicillium spp*
- *Fusarium spp*
- *Mucor spp*
- *Histoplasma*
- *Monocillium*
- *Tricoderma*
- *Chaetomium*
- *Stachybotrys spp*
- *Alternaria*
- *Phoma/Eppicoccum*
- *Pan Powdery Mildew*
- *Golovinomyces*
- *Blumeria*
- *Erysiphe*
- *Podosphaera spp*
- *Oidiodendron*
- *Rhodotorula*
- *Cladosporium spp*
- *Candida spp*

Current State of the Enviro[x] Assay

- Internal validation complete
- External AOAC surface validation in progress for highlighted organisms in an expanded Food Safety Chip

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**PathogenDx Enviro$^{X}$ Food Safety Assay – AOAC Study**

- Background on study design
- Background on expanded array content and layout
- Description of the study results

**Conclusion**

# AOAC Study Design

## Study Design Outline

1. **Inclusivity and Exclusivity Study**
   - ***Salmonella spp* –** 100 inclusive / 30 exclusive
   - ***Listeria monocytogenes* –** 50 inclusive / 30 exclusive
   - ***Listeria spp* –** 25 inclusive / 25 exclusive

2. **Matrix Study**
   - **Stainless steel, plastic, sealed concrete, and rubber**
   - **LoD –** fractional recovery (~ 1 CFU)

3. **Product Consistency and Stability**

4. **Robustness**

5. **Instrument Variation**

# Enviro[X] Food Safety Chip –Array Content

## AOAC Evaluated Array Content

- The genus ***Listeria* spp** contains 6 Species:
  - *L. monocytogenes*
  - *L. innocua*
  - *L. seeligeri*
  - *L. welshimeri*
  - *L. ivanovii*
  - *L. grayi*
- ***Listeria monocytogenes***
- ***Salmonella spp***

| | |
|---|---|
| 1 | L mono-IAP2-1.1 |
| 2 | L mono-IAP2-1.2 |
| 3 | L ivanovii -IAP1B-1.1 |
| 4 | L mono-IAP2-1.4 |
| 5 | L mono-IAP3-1.1 |
| 6 | L mono-IAP3-1.2 |
| 7 | L welshimeri - 1.1 |
| 8 | L mono-IAP3-1.4 |
| 9 | L mono-IAP3-1.5 |
| 10 | L mono-IAP3-1.6 |
| 11 | L mono-IAP3-1.7 |
| 12 | L mono-IAP4A-1.1 |
| 13 | L monB-grp2b-1.1 |
| 14 | L mono-IAP4B-1.1 |
| 15 | L mono-HLY1-1.1 |
| 16 | L monC-grp2b-1.1 |
| 17 | L mono-HLY1-1.3 |
| 18 | L welshmeri A-grp2b-1.1 |
| 19 | Lmono-PRFA1-1.1 |
| 20 | Lmono-PRFA1-1.2 |
| 21 | Listeria spp -IAP1-1.1 |
| 22 | L martii A-grp2b-1.1 |
| 23 | Listeria spp-IAP1-1.3 |
| 24 | L innocua A-grp2b-1.1 |
| 25 | Listeria spp -IAP1-1.5 |
| 26 | Listeria spp 2-16S-H3-S-J1 |
| 27 | List-grp1A-1.1 |
| 28 | List-grp1A-1.1b |
| 29 | List-grp1A-1.1D |
| 30 | Listm-grp2b-1.1 |

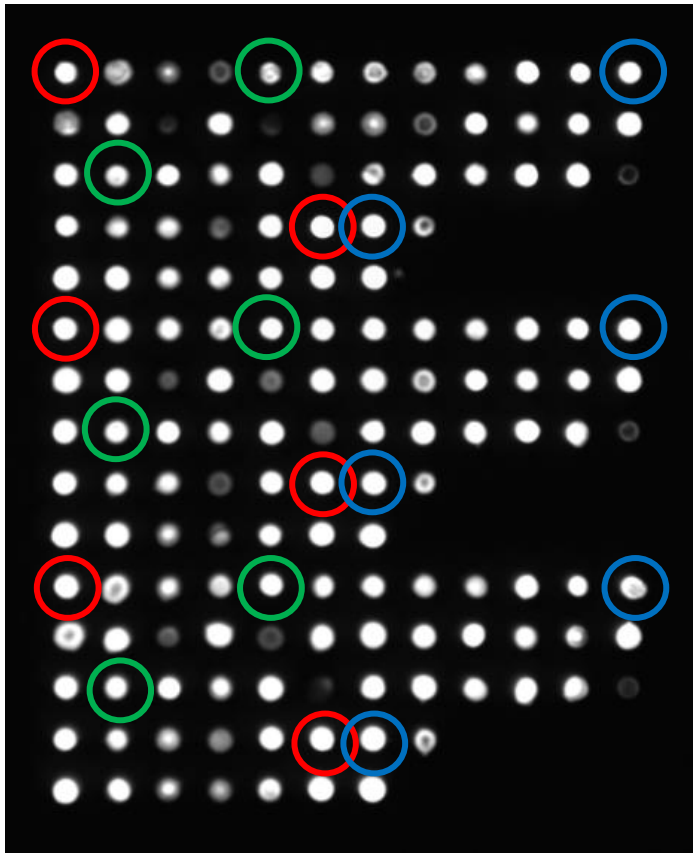
| | |
|---|---|
| 31 | **Salmonella 70-invA1-K1** |
| 32 | **Salmonella 70-invA1-I1a** |
| 33 | **Salmonella 70-invA1-I1mix** |
| 34 | **Salmonella invA2-150-1.1** |
| 35 | L ivanovii A-grp2b-1.1 |
| 36 | **Salmonella invA2-150-3.1** |
| 37 | **Salmonella invA3-700-1.1** |
| 38 | **Salmonella invA3-700-2.1** |
| 39 | **Salmonella invA3-1700-1.1** |
| 40 | L seeligeri A-grp2b-1.1 |
| 41 | **Salmonella invA3-1700-4.1** |
| 42 | Negative Control |
| 43 | Positive Control |
| 44 | Blank |
| 45 | Blank |
| 46 | Blank |
| 47 | Blank |
| 48 | Blank |
| 49 | L grayi A-grp2b-1.1 |
| 50 | L innocua -IAP1B-1.1 |
| 51 | L innocua -IAP1B-1.2 |
| 52 | L innocua -IAP1B-1.3 |
| 53 | Lmono-IAP1B-1.1 |
| 54 | Lmono-IAP1B-1.2 |
| 55 | L grayii B-grp2b-1.1 |
| 56 | Blank |
| 57 | Blank |
| 58 | Blank |
| 59 | Blank |
| 60 | Blank |

# EXAMPLE SLIDE IMAGE/MAP

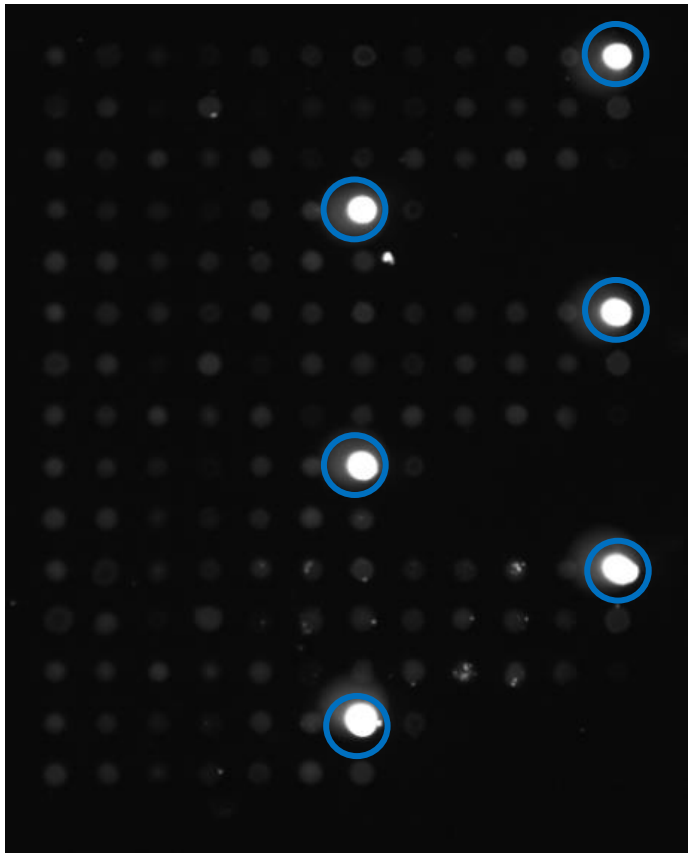
Probe - Negative Control

PCR Positive Control

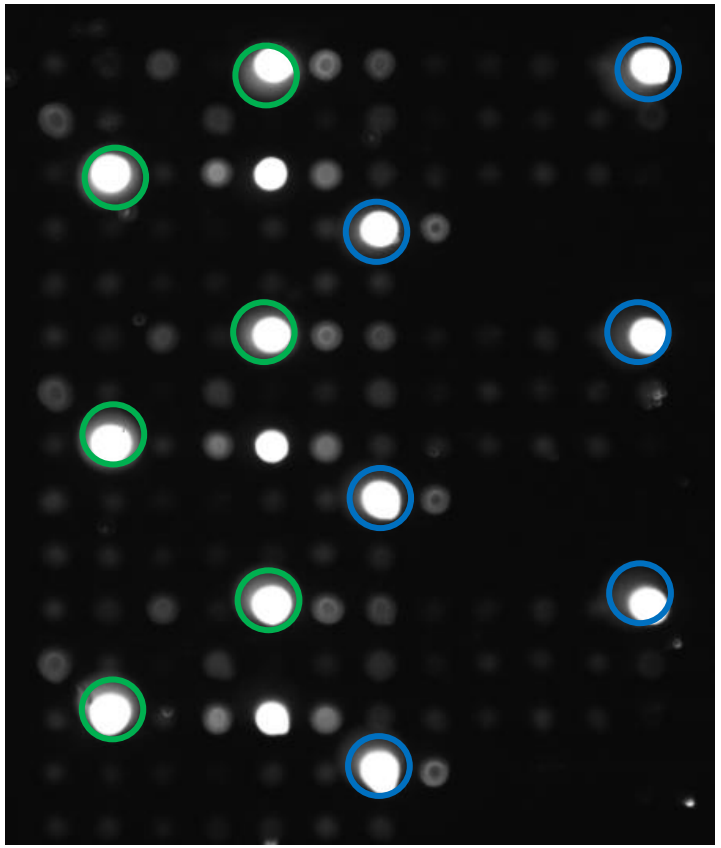
Listeria spp Probe Spot



Example Cy5 Image To show Every Spot



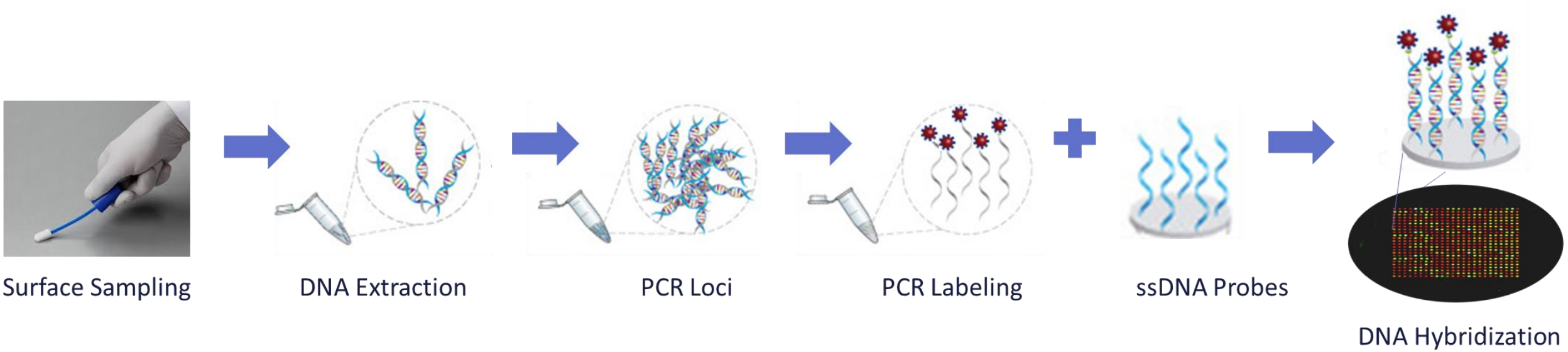
Example Cy3 Image To show just the positive control



Example Cy3 Image To show Positive Control and Listeria spp

# ENVIRO$^{X}$ MICROARRAY PROCESS DESCRIPTION

DNA microarray technology is a high-throughput diagnostic tool for the detection and identification of pathogenic organisms that can be used for direct surface sampling.



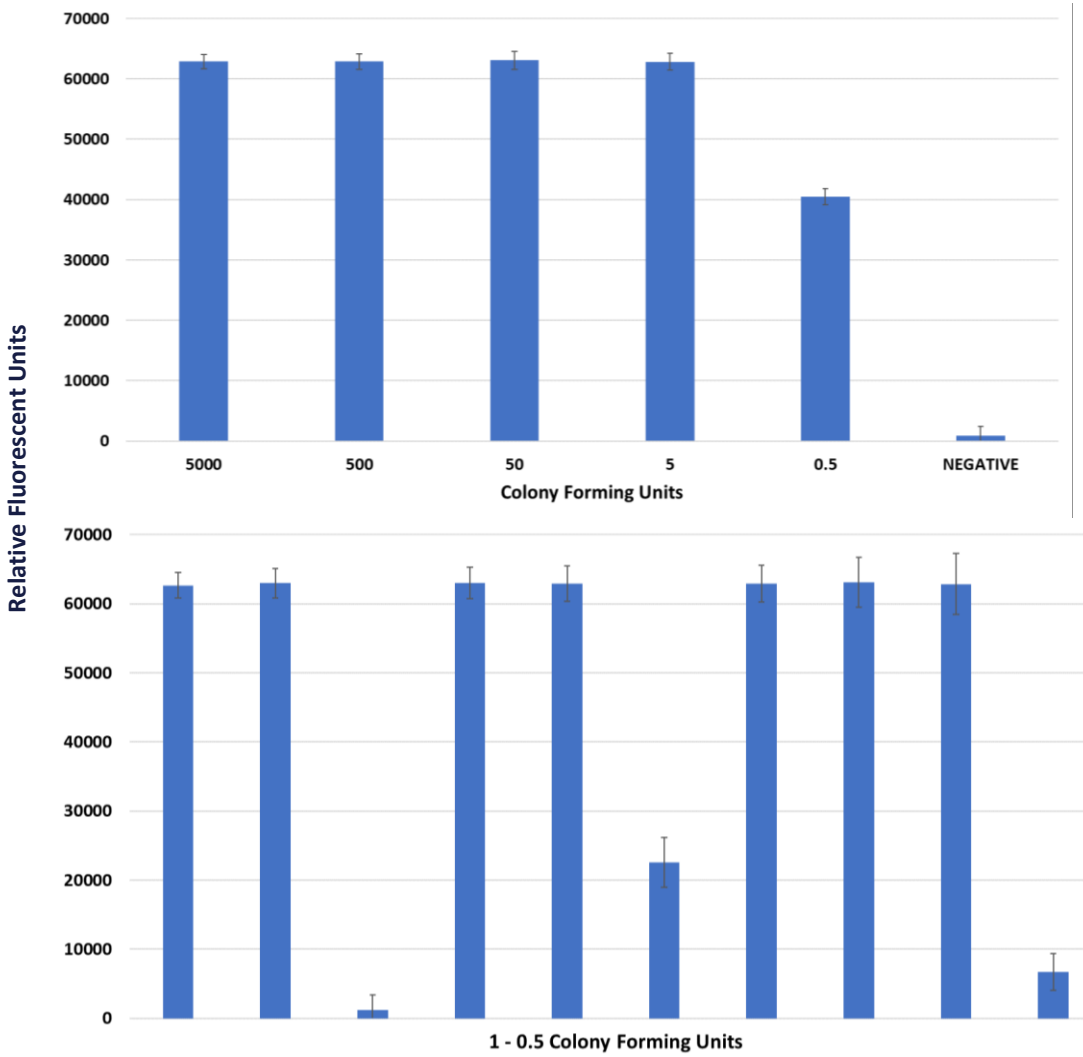

*Flexible Design, High-Throughput, Time and Cost Effective, Sensitive and Specific, without Sample Enrichment*

# LOWER LIMIT OF DETECTION PER AOAC GUIDELINES

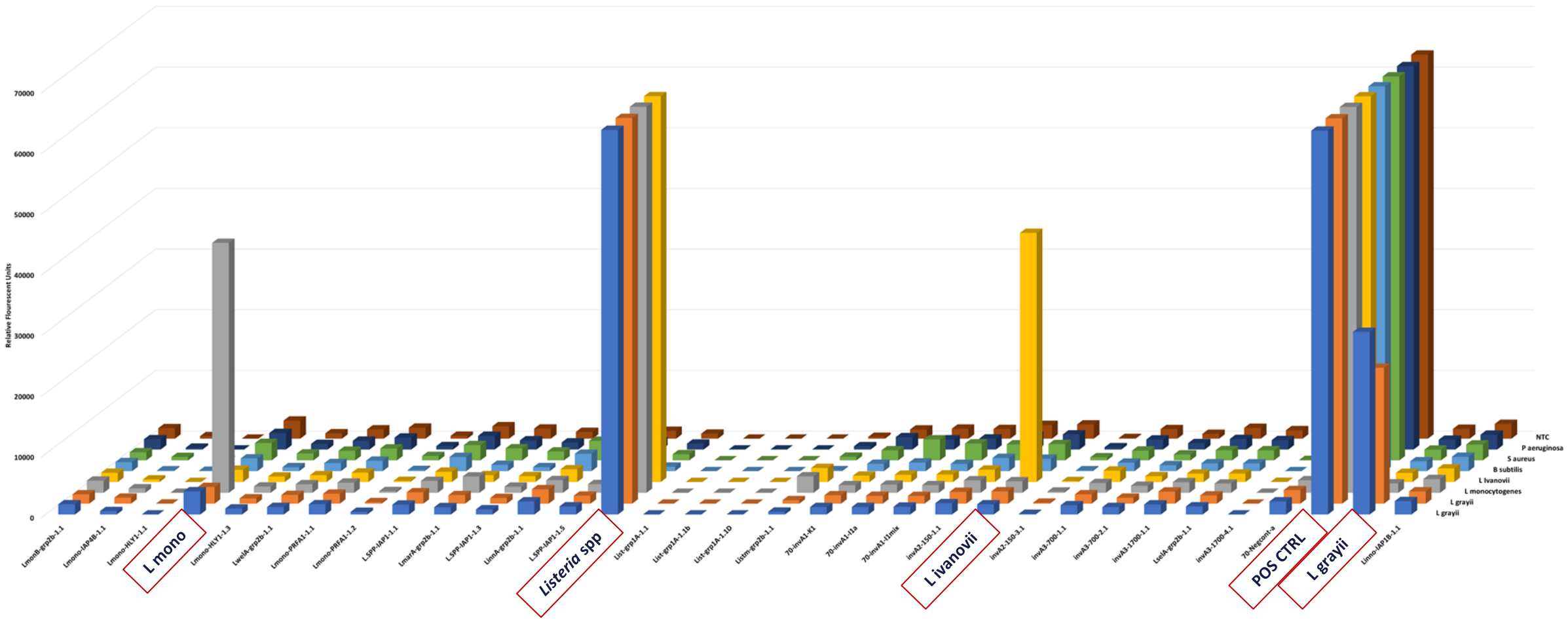
## Procedure

1. Clean stainless-steel surface with:
   - 10% Bleach and sit for 5 min
   - 70% Ethanol wipe and let the surface dry completely
   - Tape off a 4-inch x 4-inch area
2. Spot down cells:
   - Suspend the cells in media, at ~ 50x the concentration you are aiming to detect, accounting for cell death during incubation
3. Incubate the cells for 16 – 24 hours
4. Using the World Bio – PUR-Blue Hi-Cap Swab the surface in a “Z” pattern in 4 directions
5. Place the swab back into the sterile container
6. Vortex to release organisms:
   - Proceed with PDx Sample Preparation
   - Proceed with enrichment and plating

**Analytical Sensitivity of *Listeria monocytogenes* on Stainless Steel Surfaces**



# LISTERIA SPECIATION PRELIMINARY RESULTS



# Conclusion and Future Work

**Enviro[X] - Food and Environmental Safety**

- PathogenDx has created a series of multiplex diagnostic arrays for detection, quantification, and speciation
- Each detection assay has a LLOD of 1 CFU and have been developed for use in a variety of matrices
  - Food, Cannabis, Surfaces, Air
- AOAC validation study is ongoing with an expected completion date of late July

**Given the current regulatory challenges in Cannabis with microbial testing there is a need for flexible, adaptable and rapid diagnostic assays.**

- Detect[X] – Pathogenic E. coli, Salmonella enterica, Staph aureus, Pseudemonas aeruginosa, Listeria monocytogenes, and 4 Aspergillus (LLOD 1 CFU)
- Quant[X] – Broad Class Indicators (TYM, TAB, BTGN, ENT - Dynamic Range 100 – 1,000,000 CFU)
- Cannabis - Using conventional methods would require ~13 different diagnostic tests to be performed
  - Given complexity of matrices → not practical and economically sustainable for labs
- This is driving new innovative microbial diagnostics in the industry (qPCR, NGS, and DNA Microarrays)

# ANY QUESTIONS?

www.PathogenDx.com

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