

Listeria Right Now™

Environmental Listeria Test Results in Less Than One Hour

Listeria Recalls

In the News.....

2013 Listeria Outbreak

Ice Cream

3 Deaths, 10 Sick

\$2.5 to 3.5M in costs

50% workforce reduced

<https://www.nytimes.com/2015/05/08/business/blue-bell-knew-about-listeria-issues-fda-says.html>

<https://www.eater.com/2016/7/30/12332368/blue-bell-ice-cream-fine-listeria-outbreak>



2008 Listeria Outbreak

Deli Meat

24 Deaths, Many Sick

Over \$242M (CAD)

Significant layoffs

<https://www.ncbi.nlm.nih.gov/pubmed/26583272>

Listeria Recalls

In the News.....

2016 Listeria Outbreak

Salad Producing Plant

2014 had 9 different positive tests

4 month shut down

\$25.5M lost

<http://www.foodsafetynews.com/2017/05/does-springfield-shutdown-recalls-cost-company-36-million/#.Wq9SNVWnFhE>



2015 Outbreak Estimates

RTE Product

\$30 to 99M

5% cost over \$100M

Brand damage even higher costs

<https://pennstatefoodsafety.blogspot.com/2015/09/the-cost-of-foodborne-outbreaks-and.html>

FDA Listeria Recalls



2018	70
2017	108
2016	196

Directionally correct !

- Increased surveillance
- Swab-a-thons
- Test and hold strategies



Environmental *Listeria* Testing

Common Issues

"Results come back too late to do anything meaningful with them"

"It's extremely difficult to perform vectoring after positives because by the time we get back results, we've cleaned at least once or twice."

"We try to do as little Listeria testing as possible since it could be traced back to our facility via the retained culture"

"Our customers won't allow us to do in-house pathogen testing"

"We'd like to hold product pending our Listeria testing results but it costs too much in storage or our product has too short of a shelf life"

"It's too expensive to outfit a lab with all the testing equipment"



Introducing Listeria Right Now

- Complete System for *Listeria spp.*
- Molecular-level accuracy
- *No enrichment*
- Under 1 hour results



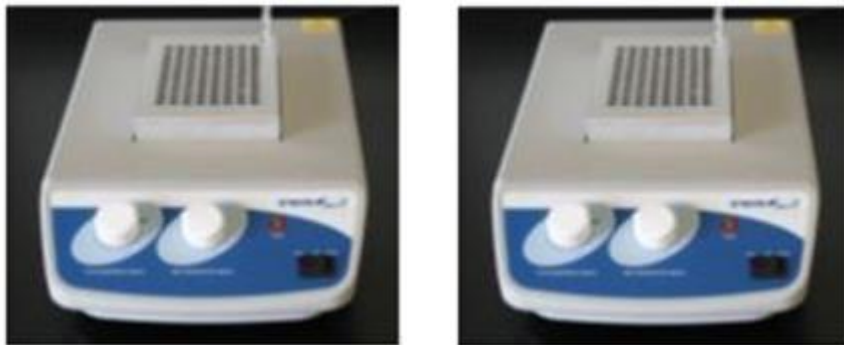
Listeria
Right Now™

System Description

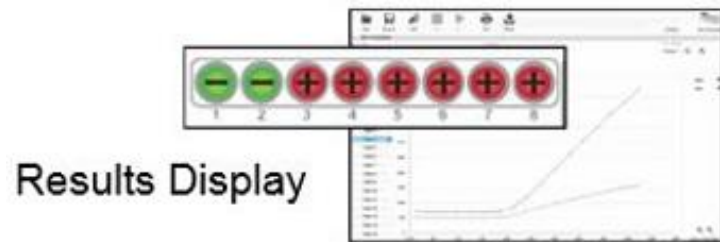
- Isothermal amplification system and reader (16 wells)
- A computer with data reporting software
- Two heater blocks - one at 37°C and the other at 80°C



System with Computer



Heater blocks

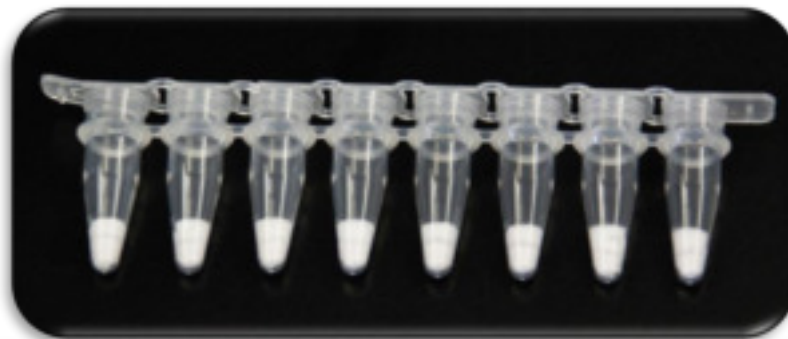


Results Display

System Description

Listeria Right Now Kit for 96 samples

- **Environmental swabs** for sample collection
- **Lysis buffer components**
- **ANSR reagents** (reaction tubes with internal positive control) *Note: the positive control is not Listeria*



A Simple Procedure

No interpretation.



Workflow Example: 6:00 a.m. Sample Collection

6:55 a.m.

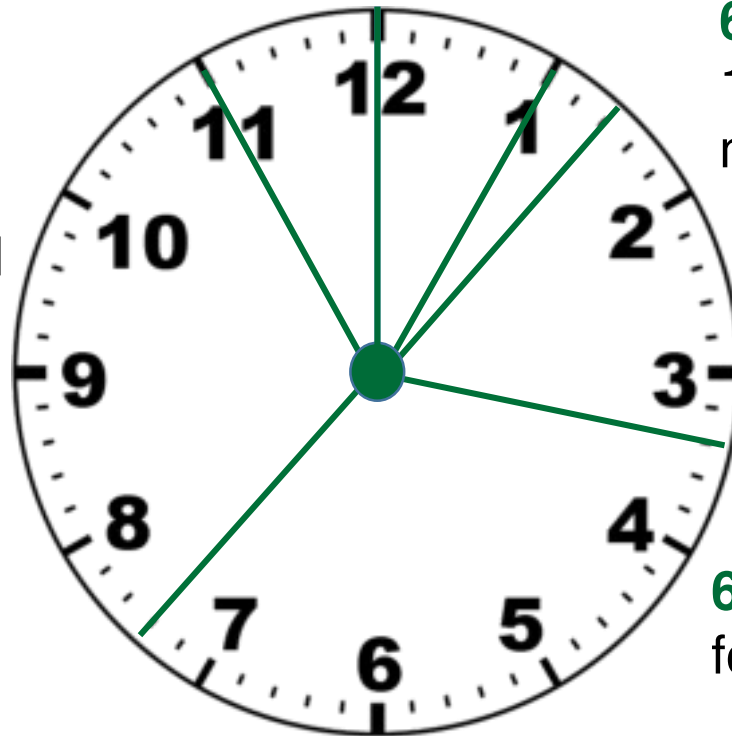
Read result

6:00 a.m. Set-up – 4 – 5 minutes

6:05 a.m. Express swab in
1 mL Lysis buffer. 2 – 3
minutes

6:07 a.m. Incubate at 37°C
for 10 minutes

6:17 a.m. Incubate at 80°C
for 20 minutes



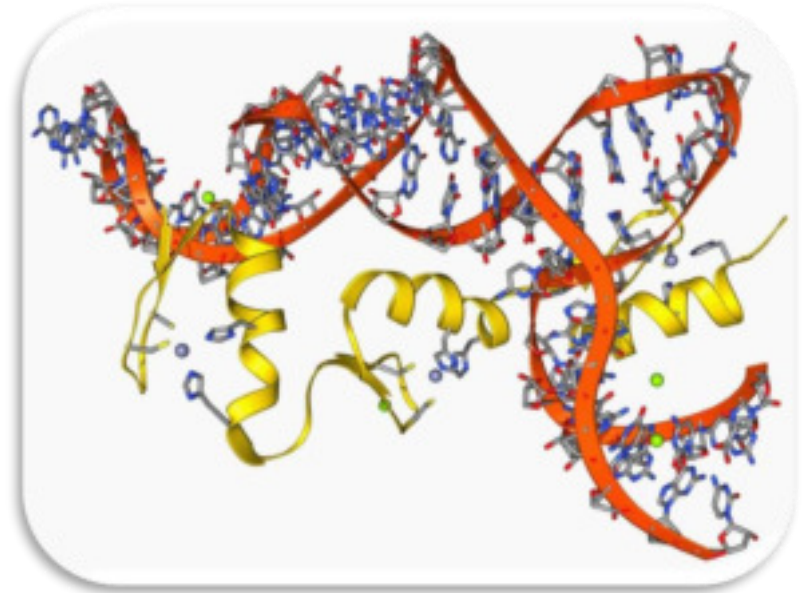
6:37 a.m.

Transfer 50 μ L to
reaction tube and
run on ANSR
reader - 18
minutes

6:56 a.m. Begin operations or re-clean and retest

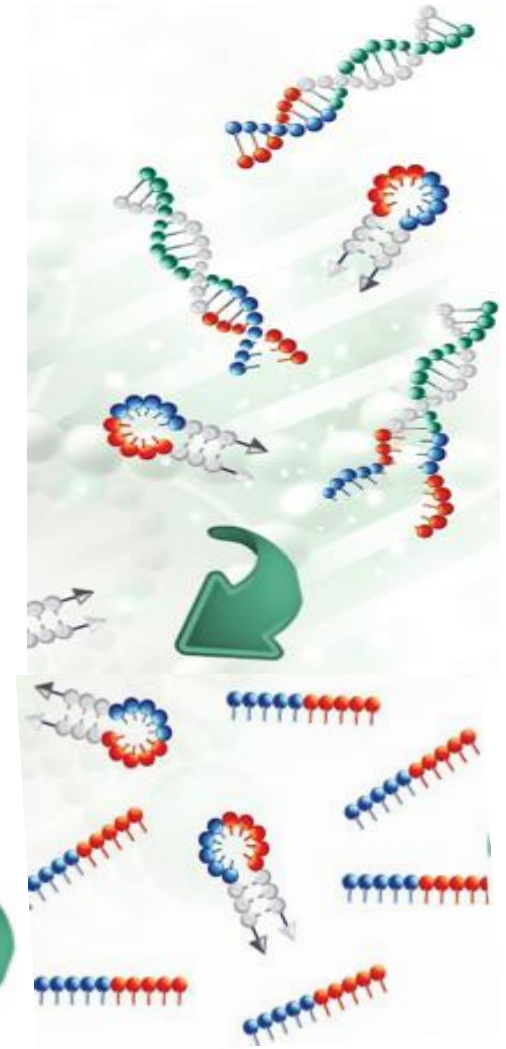
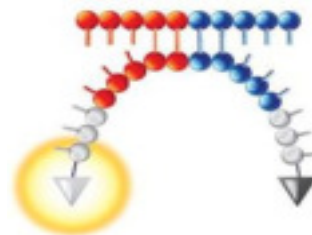
How is This Possible?

1. Ribosomal RNA is present in much greater numbers in *Listeria* cells than a traditional DNA target (~1000 – 10,000 copies per cell Vs 1 copy per cell for DNA). This can result in a 1,000 – 10,000 fold increase in target analyte concentration.



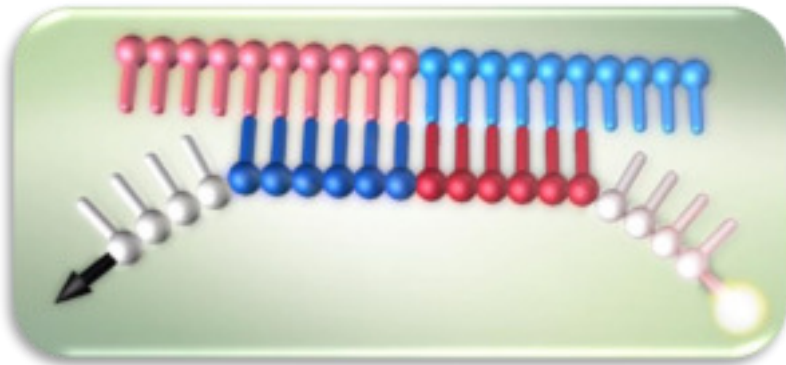
How is This Possible?

2. The isothermal reaction within the ANSR instrument produces a constant cycle of molecular replication producing analyte copies much more quickly than traditional PCR reactions which run through a series of heating and cooling cycles.



How Is This Possible

3. Detection occurs in real-time using a special molecular beacon which causes fluorescence to be detected in the ANSR instrument



How is This Possible?

Summary

starting with significantly
more targets

+ a significantly faster cycle
time

= significantly faster results.



Data – Internal Env. Surface Study

Results and probability of detection calculations for the *Listeria* Right Now presumptive and culture confirmation assays

Surface type	Trial	Listeria CFU/swab	N	LRN +	Culture +
Stainless steel	<i>Lm</i> (4b) only	1	15	3	2
		2	15	10	7
		2438	5	5	5
		0	5	0	0
Stainless steel	<i>Lm</i> (4b) + background	1.8	20	8	7
		1800	5	5	5
		0	5	0	0
Plastic	<i>L. innocua</i> + background	2.3	20	9	9
		2250	5	5	5
		0	5	0	0
Sealed concrete	<i>L. welshimeri</i> + background	1.2	20	6	11
		1550	5	5	5
		0	5	0	0
Ceramic tile	<i>Lm</i> (1/2a) + background	1.93	20	14	9
		1930	5	5	5
		0	5	0	0

Listeria Right Now has been validated on multiple environmental surfaces



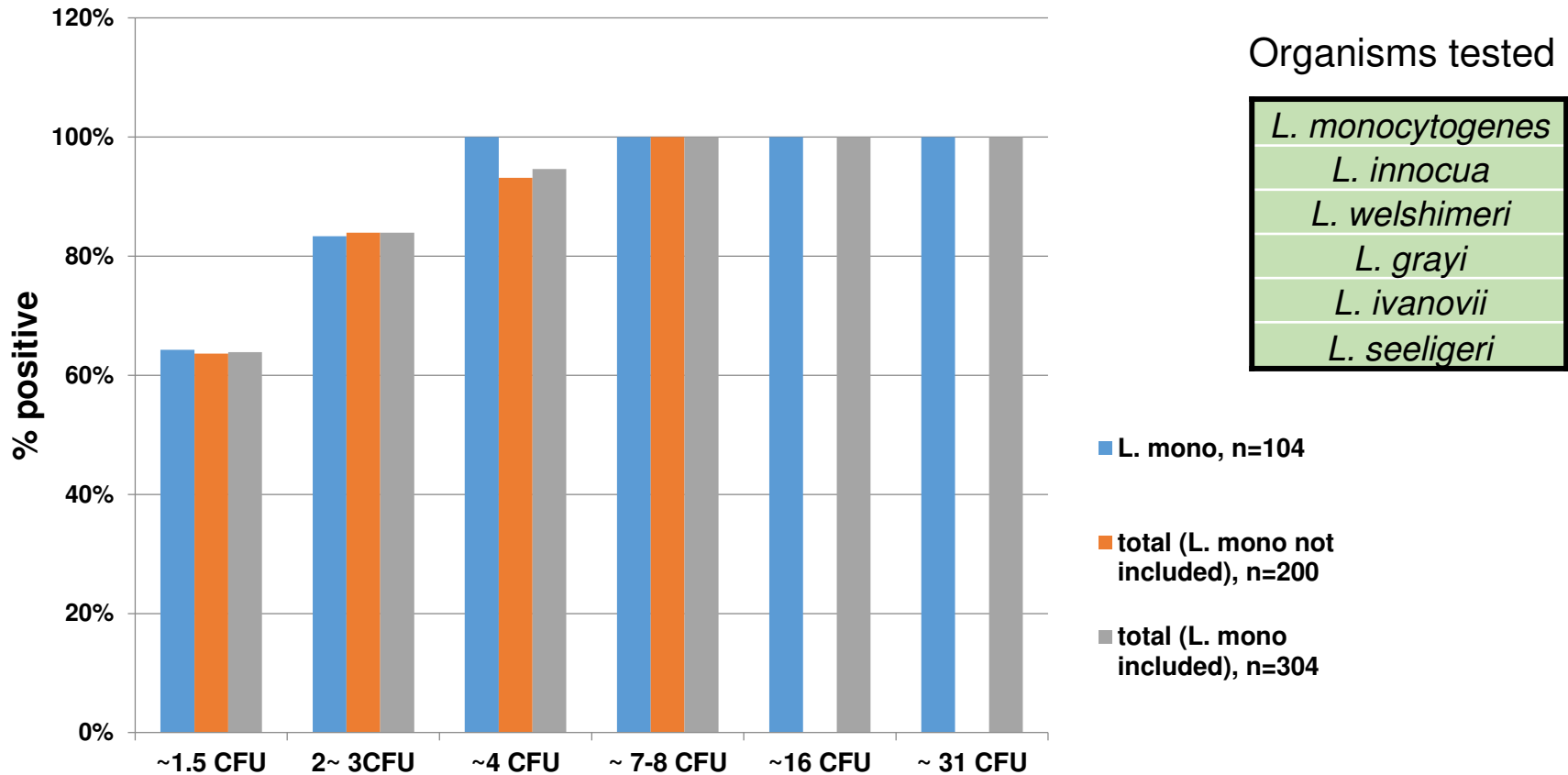
Data -Testing with Sanitizers

Effects of Food Industry Sanitizers/Disinfectants on *Listeria* Right Now Assay

Sample ID	# swab	LRN assays	positive	negative	invalid
No <i>Listeria</i> organism on the surface					
10% bleach	7	14	0	14	0
Mandate Plus	7	14	0	14	0
Ster-Bact	7	14	0	14	0
Peroxyacetic acid 1%	4	4	0	4	0
Peroxyacetic acid 2%	4	4	0	4	0
negative control	3	6	0	6	0
With <i>Listeria</i> spp. on the surface					
XY-12	4	8	8	0	0
Mandate Plus	4	8	8	0	0
Peroxyacetic acid 1%	4	4	4	0	0
Peroxyacetic acid 2%	4	4	4	0	0
Water control	4	8	7	1	0

Conclusion: No interference from residual sanitizers

Data - Limit Of Detection



Theoretical limit of detection = 2 CFU / swab

95% Confidence Interval L.O.D. = 4 CFU / swab

Customer Feedback

With *Listeria* Right Now we can:

“Expand testing to include zone 1 areas and reclean same-shift”

“Perform a corrective action much more quickly and fix an issue before it becomes a serious problem.”

“Be more flexible and proactive with our sampling program. A faster time to result expands the usefulness of the system.”

“Can now test & hold.”

“Save a LOT of money on outside testing”

“Reduced testing turnaround time from 48 or 72 hours to one hour”



Thank You

*See You At The
Information Open House*