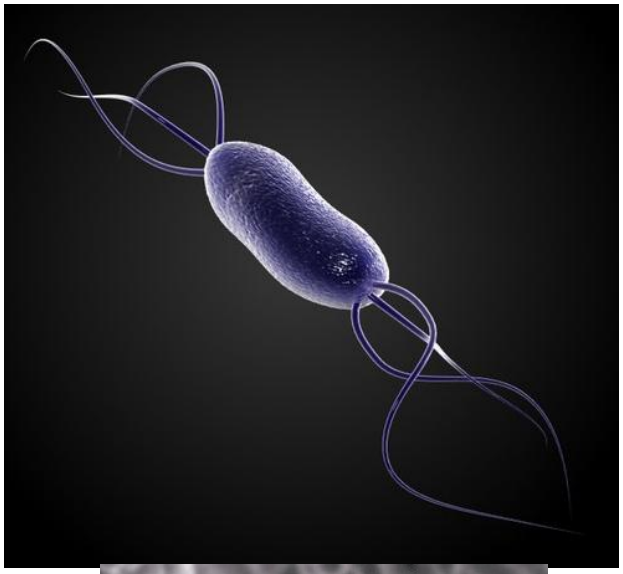
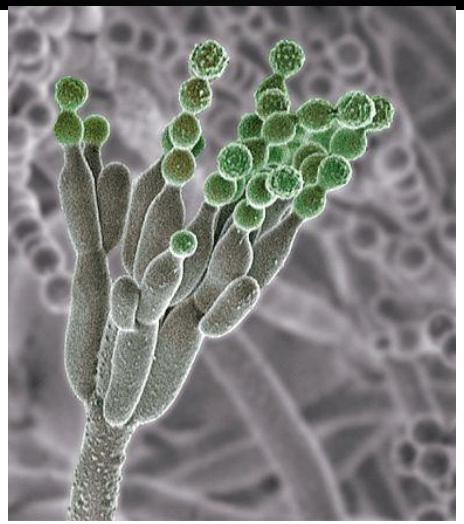




Neogen Rapid Listeria Solutions:

ANSR -More Sensitive than ISO

Listeria Right Now – Results in 1 hour

Antonio Debán Valles

Microbiology Application Specialist

Neogen Corporation

The World's leading Animal and Food Safety Company

Founded: 1982

Nasdaq: NEOG

Locations: Michigan, Kentucky, Nebraska, Wisconsin, Mexico, Brazil, China, India, England & Scotland

Employees: 1100+

Market Capitalisation: $2B


Neogen Canada
Guelph, ON
Neogen Europe
Ayr, Scotland
Lab M
Heywood, England
Quat-Chem
Rochdale, England
Neogen China
Shanghai, China
Neogen Latinoamerica
Mexico City, Mexico
Corporate Headquarters
Food Safety Division,
Lansing, MI
Animal Safety Division
Lexington, KY
Hacco, Inc.
Randolph, WI
GeneSeek
Lincoln, NE
Prima Tech
Kenansville, NC
Chem-Tech
Pleasantville, IA
Preserve International
Memphis, TN
Turlock, CA
Neogen India
Cochin, India
Neogen do Brasil
Sao Paulo, Brazil
Deoxi Biotecnologia
Araçatuba, Brazil
Rogama
Pindamonhangaba, Brazil

NEOGEN

DCCASA

Animal Health

- Public Animal Health Labs
- Veterinary Pharma Industries
- Animal Production Industries
- Veterinary Universities
- Private External Veterinary Labs

Food Quality Control

- Food Industry
- Public Health Institutes
- Food Technology Universities
- Private External QC Labs
- Dairy QC Labs

nirco
Diagnóstico & Investigación

NEOGEN

Neogen Throughout the Food Chain

Food Safety Solutions

General Micro & Sterility Testing

In Process/Process Control

Environmental Monitoring

Speciation & Adulteration

NEOGEN®

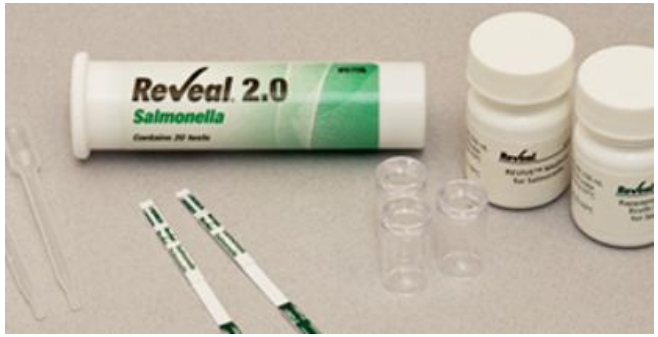
Pathogens Finished/Packaged Products

Raw Materials

NEOGEN®

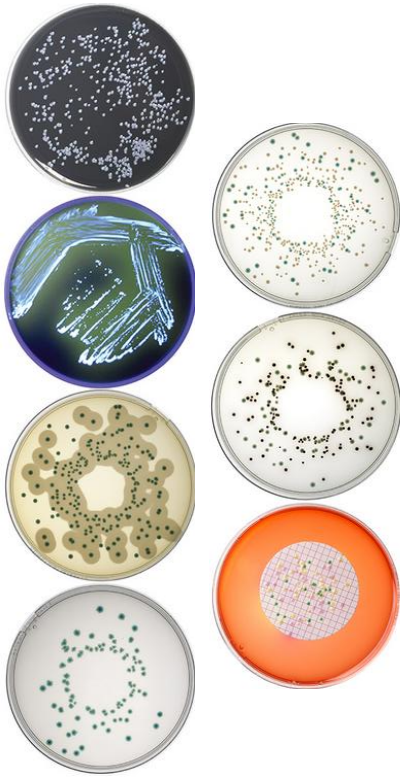
Neogen – Rapid pathogen detection

Reveal 2.0



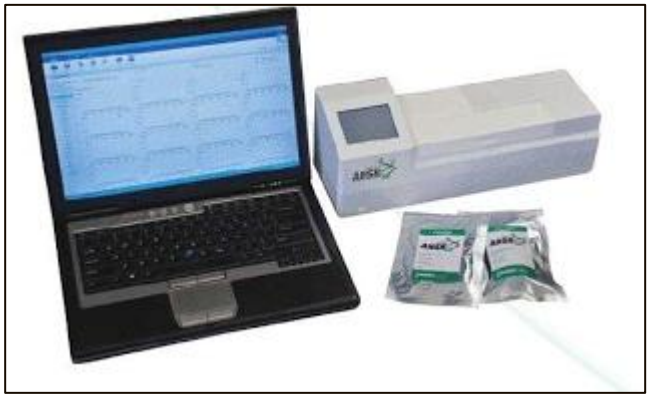
NEOGEN®
ANSR™
Pathogen Diagnostics

LAB™
A Neogen® Company



NEOGEN

ANSR footprint



New molecular methods: ANSR

Amplified Nucleic Single-Temperature Reaction

For rapid detection of pathogens

New Technology

- Based on **isothermal DNA amplification**
- Genetic level detection of RNA and DNA
- Highly Sensitive

Simple

3 steps:

Enrichment, Lysis and Amplification/Reading

Faster

- Repeated-thermocycling not required
- **Amplification/Reading 10 to 18 min**
- Results in as little as 24 hours

Minimal cost and footprint

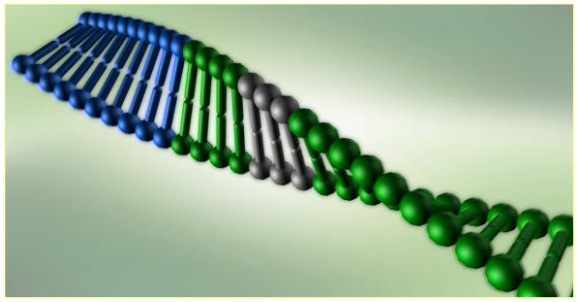
- Compact size to easily fit in any lab
- Instrumentation not expensive

User Friendly

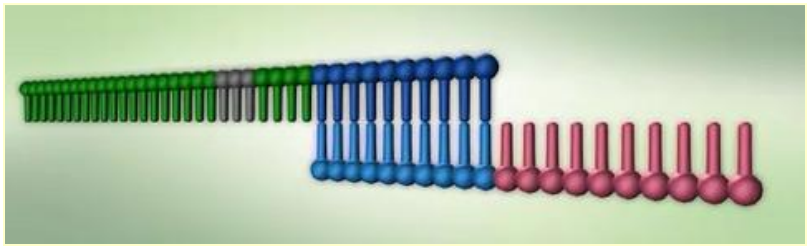
- Easy to perform
- Easy to see results, record and report

NEOGEN

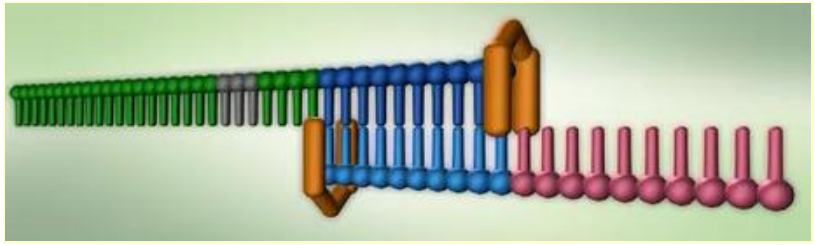
How does ANSR work?



DNA is released through lysis of the bacterial cells in the enriched sample

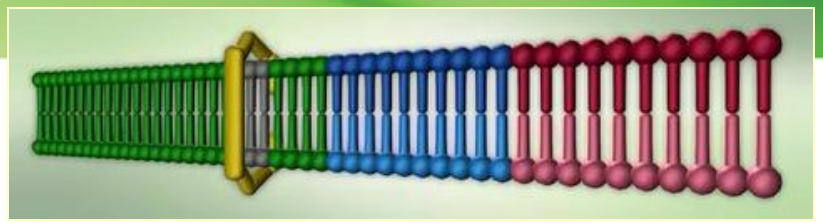


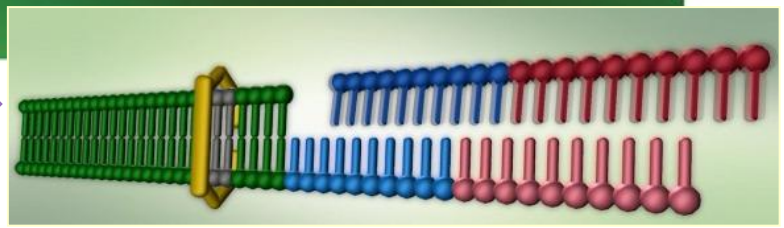
A small oligonucleotide primer binds to the complementary pathogen DNA



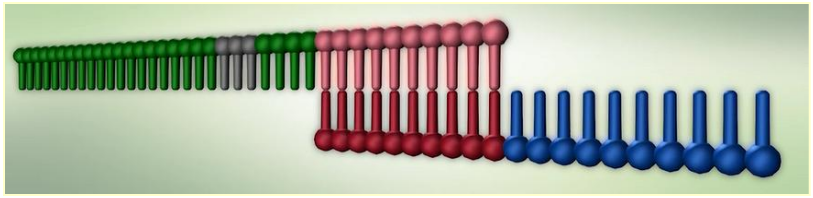
A DNA polymerase recognizes the overhang as being damaged and extends the nucleotides along the strand creating a new piece of DNA

NEOGEN

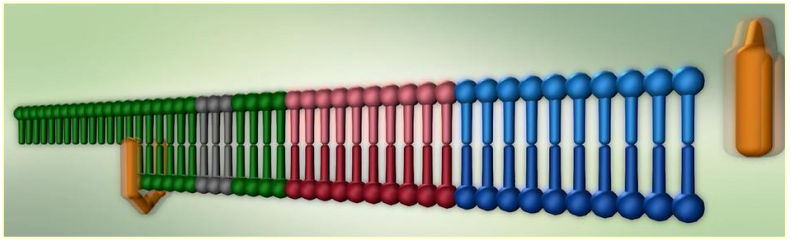




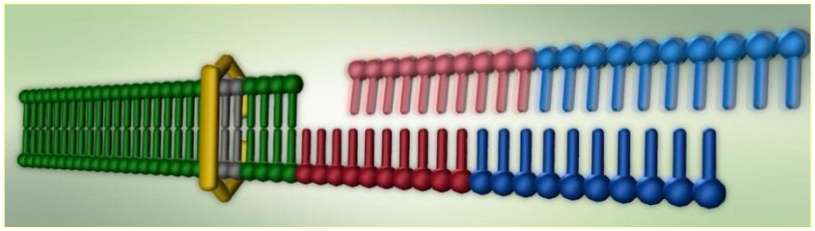
A nicking enzyme “cuts” through one strand of the DNA releasing the fragment



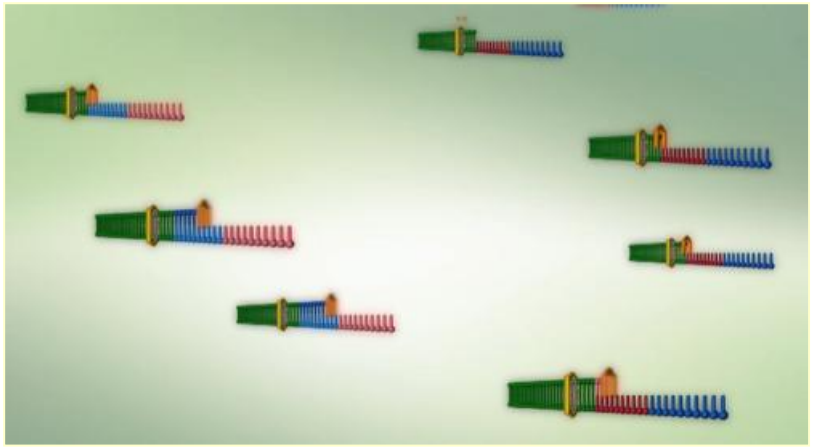
The product from the first reaction binds with a second primer



A DNA polymerase again extends the nucleotides creating a new piece of DNA



A nicking enzyme “cuts” through one strand of the DNA releasing the fragment

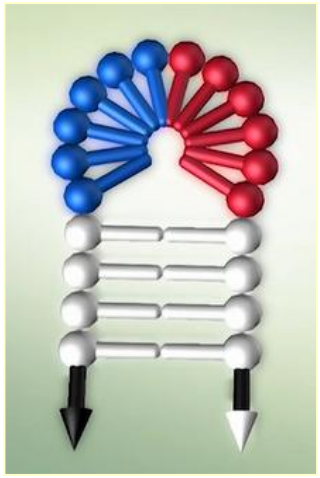


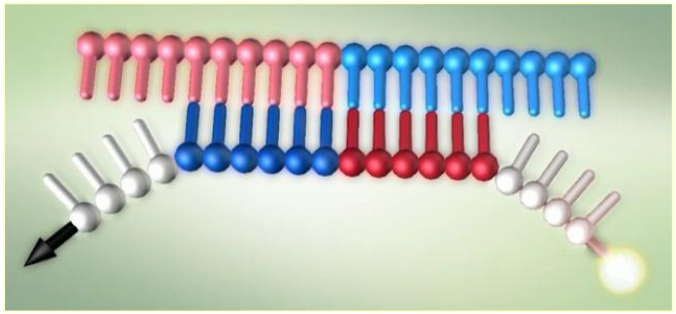
NEOGEN

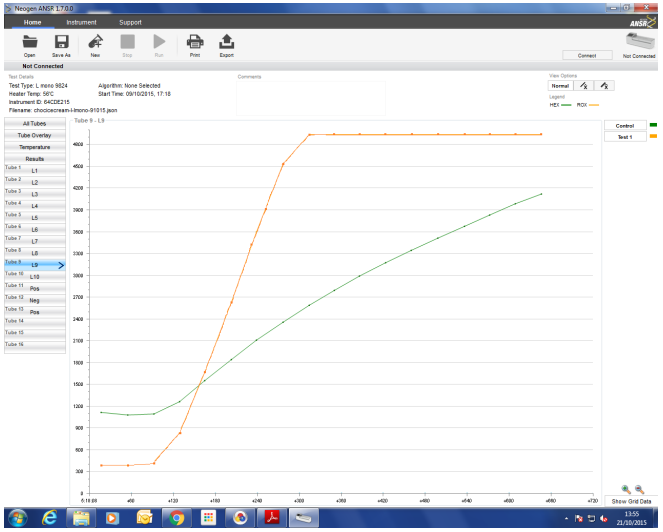
How does ANSR work?

- The amplified segments of the pathogen DNA attach to special molecular beacons

- The molecular beacons fluoresce when bound to the pathogen DNA. This is detected by the ANSR reader.







ANSR compared to PCR amplification

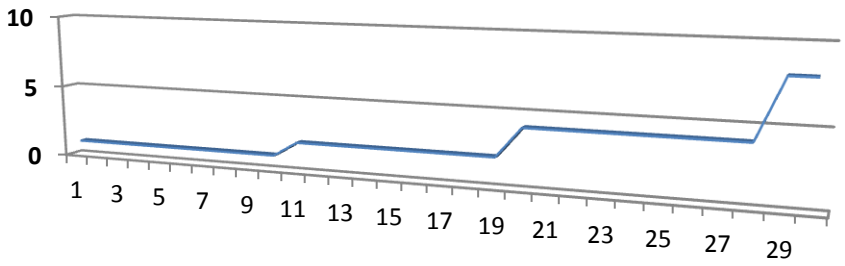
Polymerase **C**hain **R**eaction - PCR

- A single or a few copies of a piece of DNA have to be amplified through thermo-cycling to generate thousands to millions of copies of a particular DNA sequence.
- Repeated cycles of denaturation and polymerisation are required.
- Exponential replication occurs once each cycle.

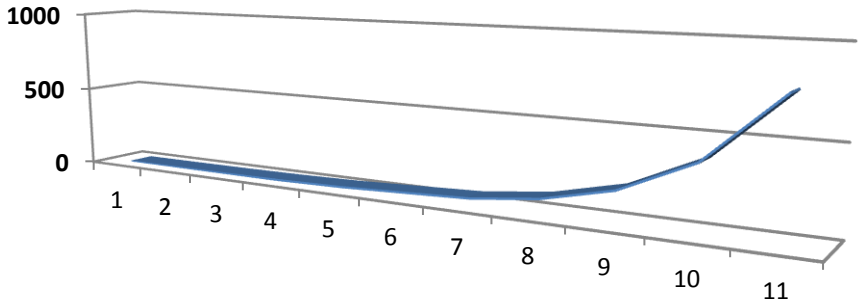
Amplified **N**ucleic **S**ingle-Temperature **R**eaction - ANSR

- Exponential, continuous isothermal chain reaction in which the product of one reaction catalyses further reactions.
- Repeated thermo-cycling not required.

PCR Cycle Replication

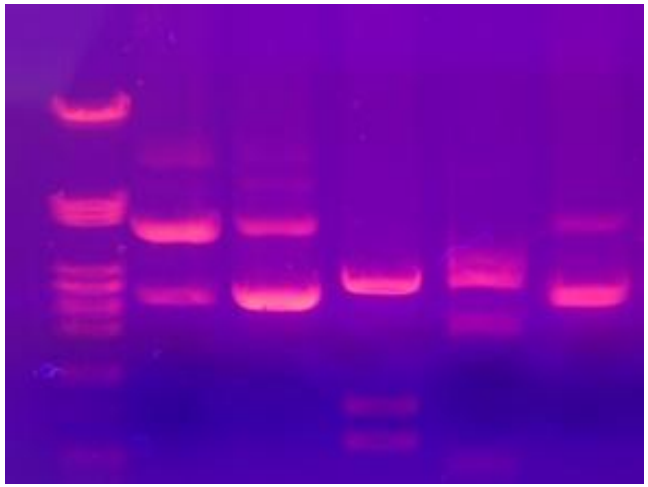


ANSR Continuous Replication

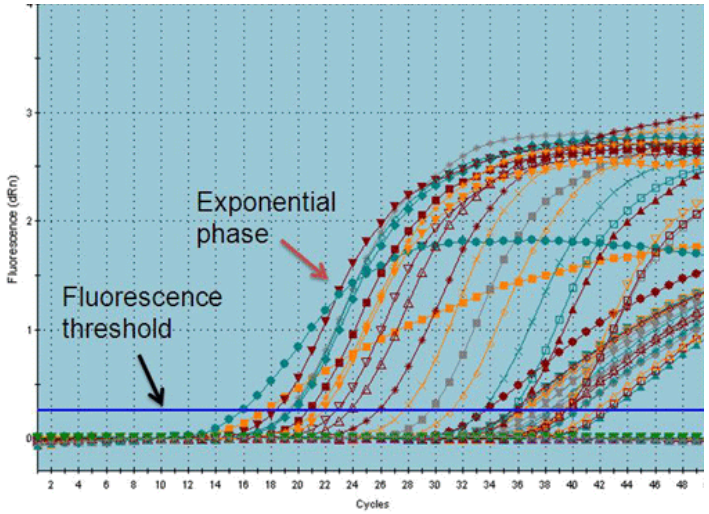


ANSR compared to PCR- results

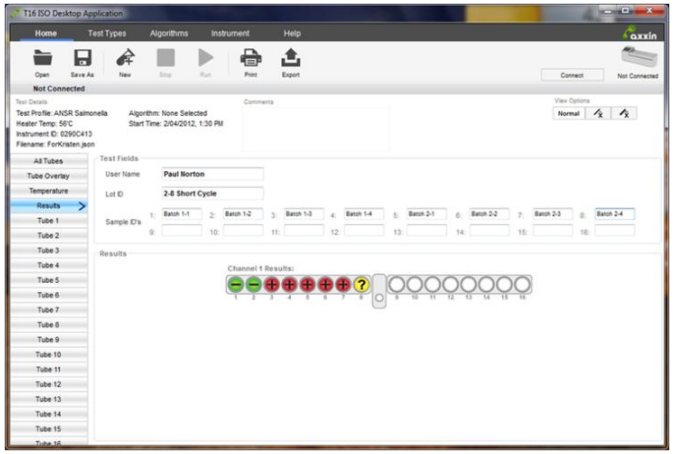
Traditional PCR

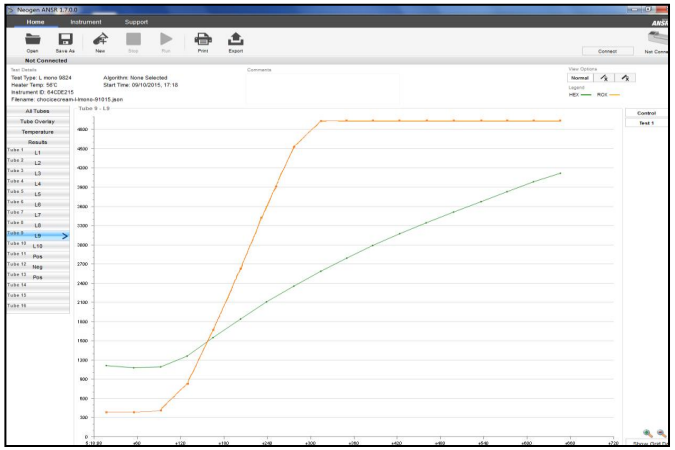


rt-PCR



ANSR





ANSR compared to PCR – time to results

MOLECULAR REACTION

Traditional PCR: Several hours

rt-PCR: Up to 1 hour

ANSR: 10 – 18 minutes

WHOLE PROCESS

Traditional PCR: Up to 30h

rt-PCR: As little as 26h

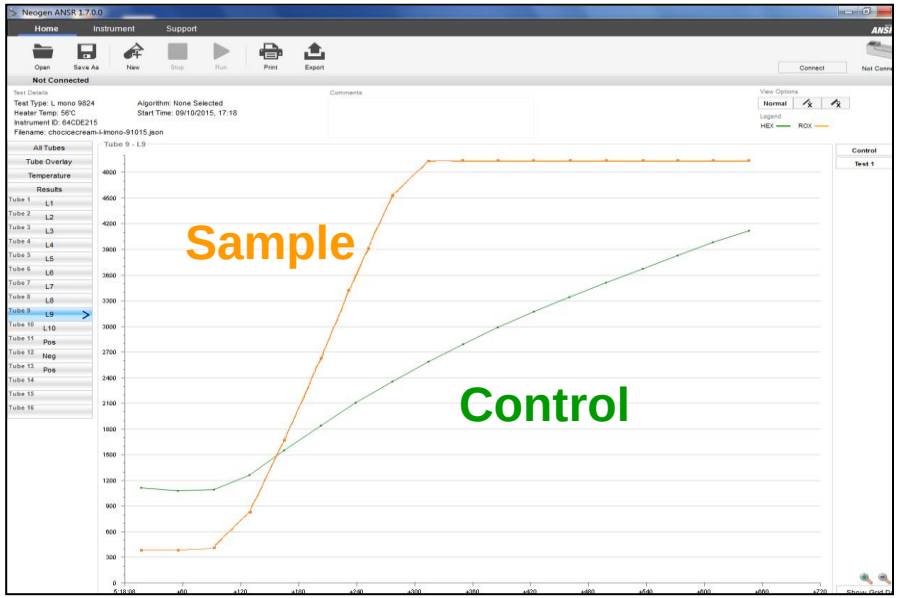
ANSR: As little as 24h

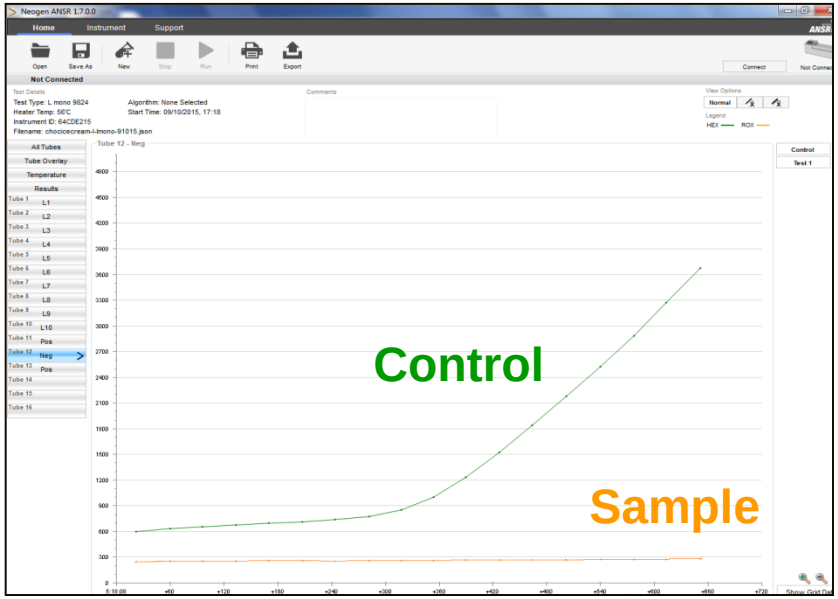
The importance of an internal control

It clearly tells the user if inhibition has occurred during the analyses. Several food products may contain compounds causing inhibition.

A dye called SYTO 82 inserts itself into double-stranded DNA giving off a signal at a different wavelength than the beacon

The signal may be stronger in positive samples because there are more chances for the templates and products to bind to each other





ANSR solutions

- Simplified, single step enrichment
- Applicable to food and environmental sample matrices

ANSR *Salmonella*

- Sensitivity: 1 cfu/analytical unit
- Sensitivity: 10^4 cfu/mL post enrichment
- Testing time: 10 minutes
- Based on DNA

ANSR *Listeria* spp

- Sensitivity: 1 cfu/analytical unit
- Sensitivity: 10^2 cfu/mL post enrichment
- Testing time: 18 minutes
- Based on RNA

ANSR *E. coli* O157:H7

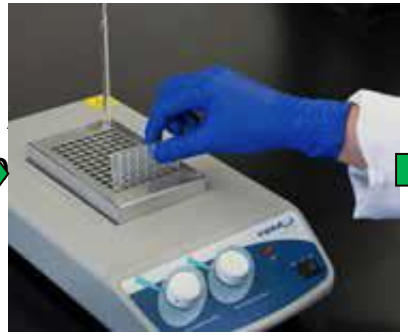
- Sensitivity: 1 cfu/analytical unit
- Sensitivity: 10^4 cfu/mL post enrichment
- Based on DNA

ANSR *Listeria monocytogenes*

- Sensitivity: 1 cfu/analytical unit
- Sensitivity: 10^4 cfu/mL post enrichment
- Testing time: 10 minutes
- Based on DNA

NEOGEN

Simple workflow for all methods

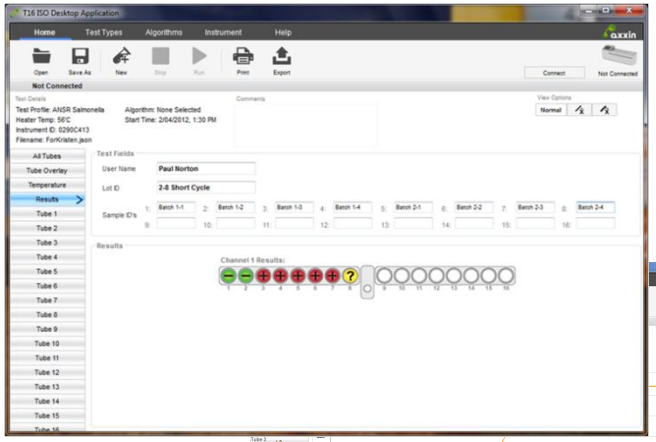


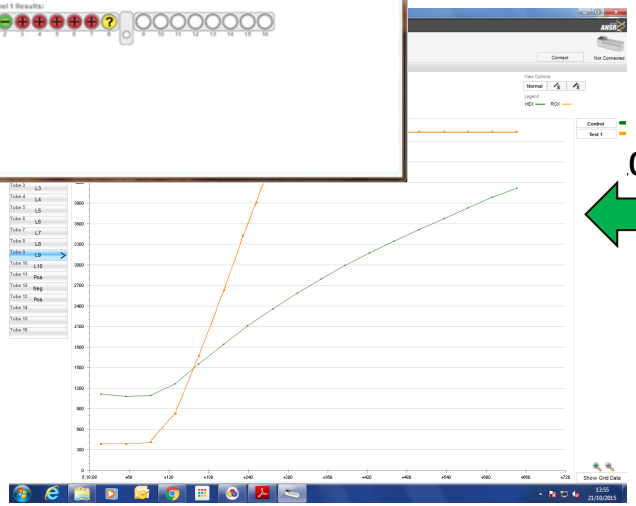
80ºC for 20

Transfer 50ul to

der

ex

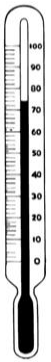




Results in as little as 24 hours from start of enrichment to results

NEOGEN

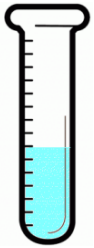
Protocol for single samples



24+/-2h * in LESS+ broth at 30°C

*For *Listeria* spp.
24±2h
*For *L. monocytogenes*
26±2h

50µL of sample



Add 450 µL of lysis buffer to the sample.

37°C heat block
10 minutes.
80°C heat block
20 minutes.

Transfer 50 µL to preheated lyophilized reagents (56°C) in the reader.
Cap tubes and vortex briefly.
Return tubes to reader Start.
Results in 10 minutes

BY AFNOR CERTIFICATION
NF VALIDATION EN ISO 16140

NEO 35/03-01/16
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
http://nf-validation.afnor.org/en

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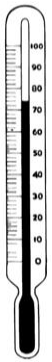
Protocol for pooling samples



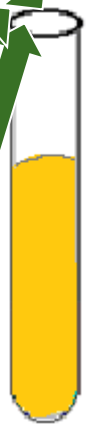
24+/-2h * in LESS+ broth at 30°C

From 1 to 10 samples

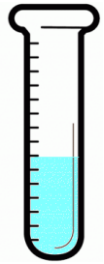
1mL

1mL

1mL



50µL of sample



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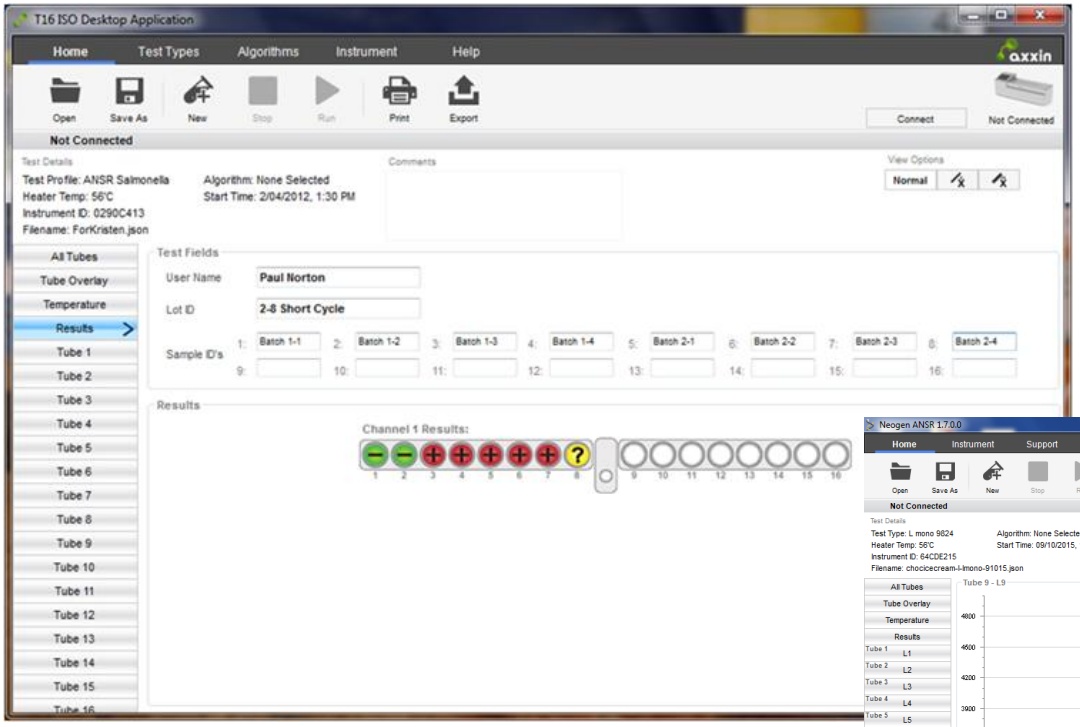
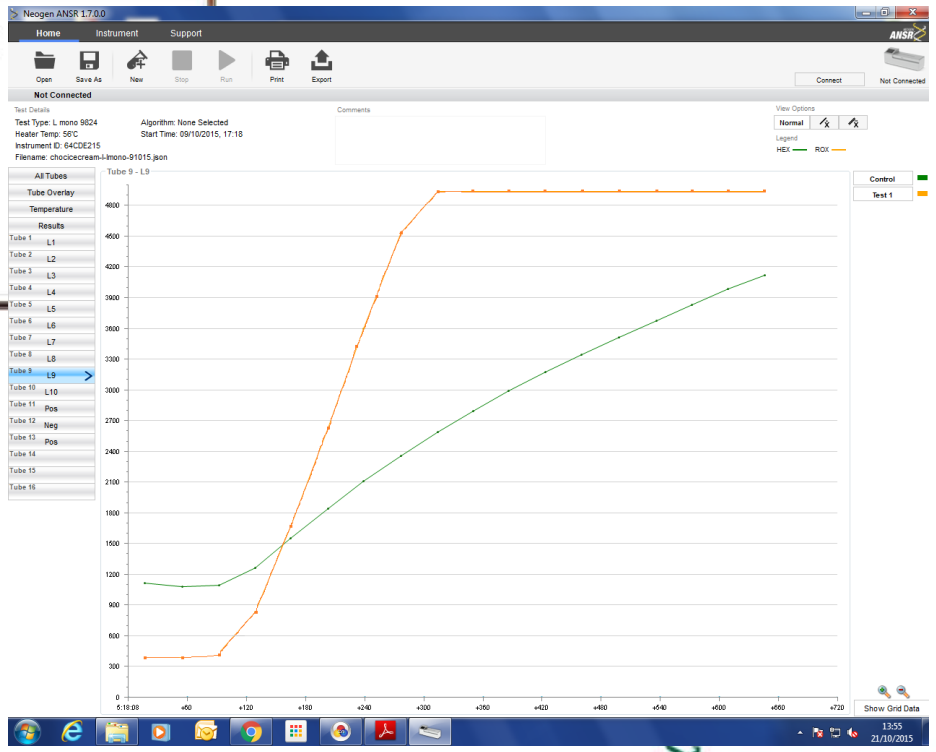
BY AFNOR CERTIFICATION
NF VALIDATION
EN ISO 16140

NEO 35/03-01/16
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
http://nf-validation.afnor.org/en

•For *Listeria* spp. 24±2h
•For *Listeria mono.* 26±2h

NEOGEN





A positive result will need to be confirmed

ANSR ISO 16140 Validations

- ANSR *Listeria spp* results

i. Pooled samples

	ISO +	ISO -
ANSR +	119	**38**
ANSR -	**31**	261

Δ =7

ANSR *Listeria* is more sensitive than ISO

ii. Single

	ISO +	ISO -
ANSR +	120	**40**
ANSR -	**30**	259

Δ =10

ANSR *Listeria* spp - Sensitivity

Sensitivity = (positive with the method)
/(Total positive results)

Sensitivity	Pooling	Single
ANSR *Listeria*	83.5 %	84.2 %
Reference	79.8 %	78.9%

What is new?

Listeria RIGHT NOW

ANSR solutions – *Listeria* Right Now

An enrichment-free environmental monitoring tool for *Listeria* spp detection providing results in less than 60 minutes

ANSR solutions – *Listeria* Right Now

Results in under an hour, how is it possible?

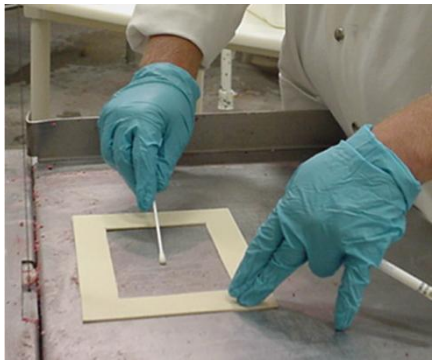
No pre-enrichment needed

Only two heating steps: 10 min at 37°C + 20 min at 80°C

ANSR *Listeria* spp reaction: 18 min

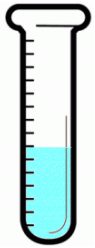
Protocol for LRN

Introduce swab



1mL of buffer
Swirl swab

500µL



37°C heat block
10 minutes.
80°C heat block
20 minutes.

Transfer 50 µL to preheated lyophilized reagents (56°C) in the reader.
Cap tubes and vortex briefly.
Return tubes to reader Start.
Results in 10 minutes

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ANSR solutions – *Listeria* Right Now

Validation study – Neogen Corporation

- Surfaces tested during study:

Stainless steel

Sealed concrete

Plastic

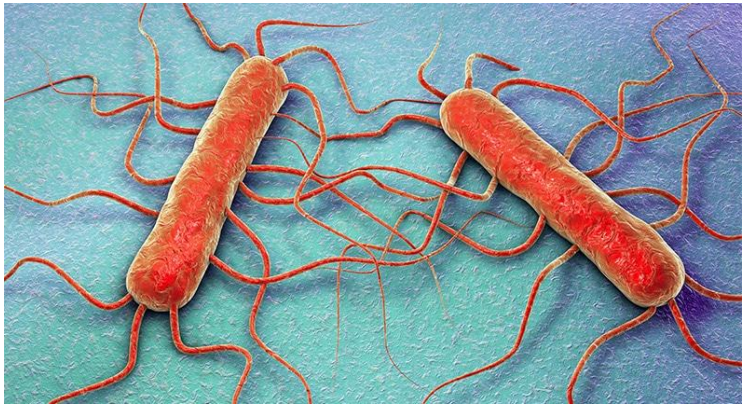
Ceramic tiles

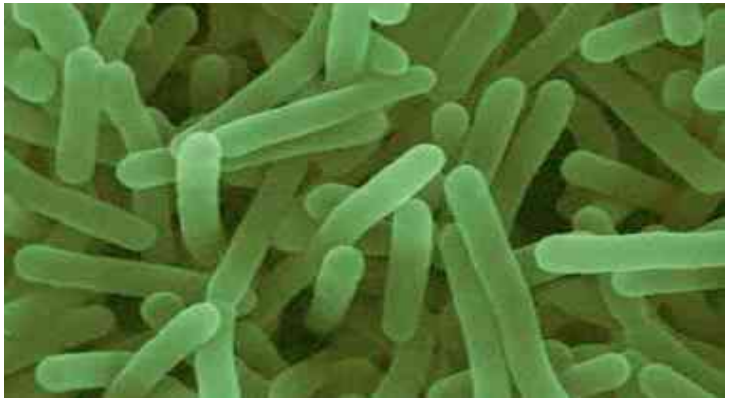
NEOGEN

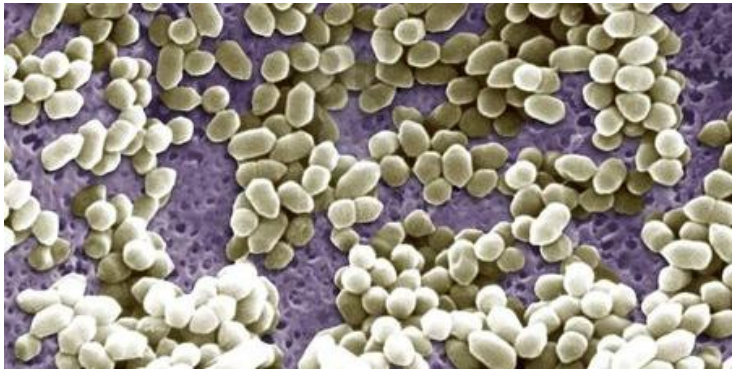
ANSR solutions – *Listeria* Right Now

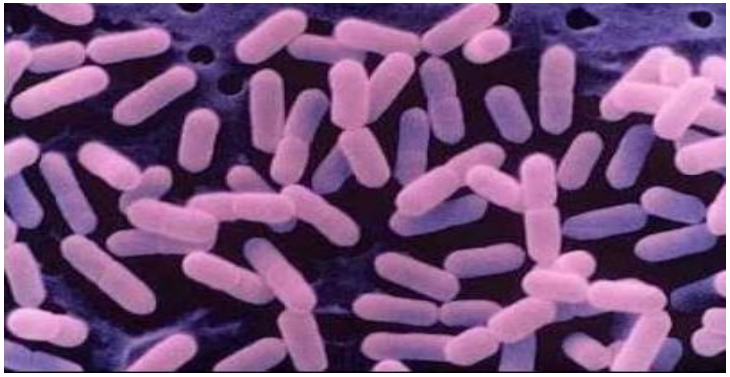
Validation study – Neogen Corporation

- Different *Listeria* spp strains tested: *L. monocytogenes*, *L. innocua*, *L. ivanovii*, *L. grayi, L. seeligeri*, *L. welshimeri*







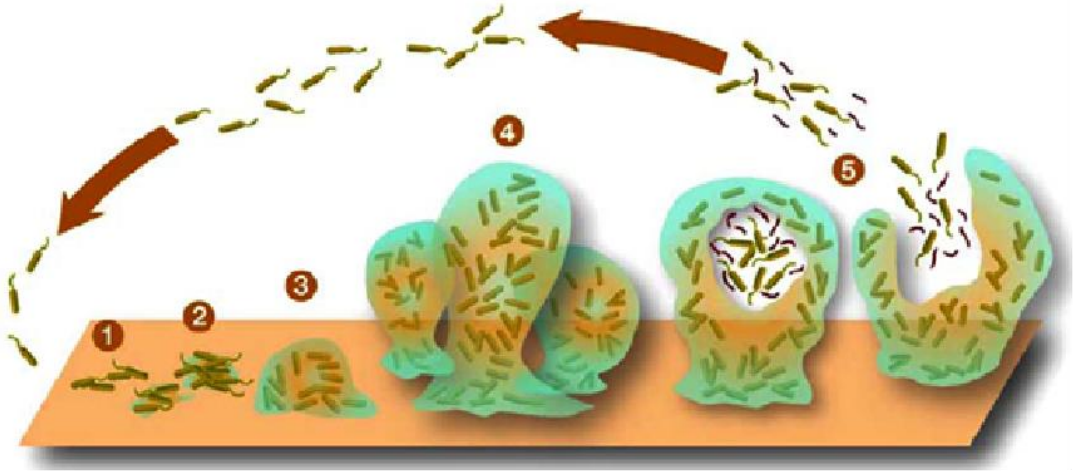


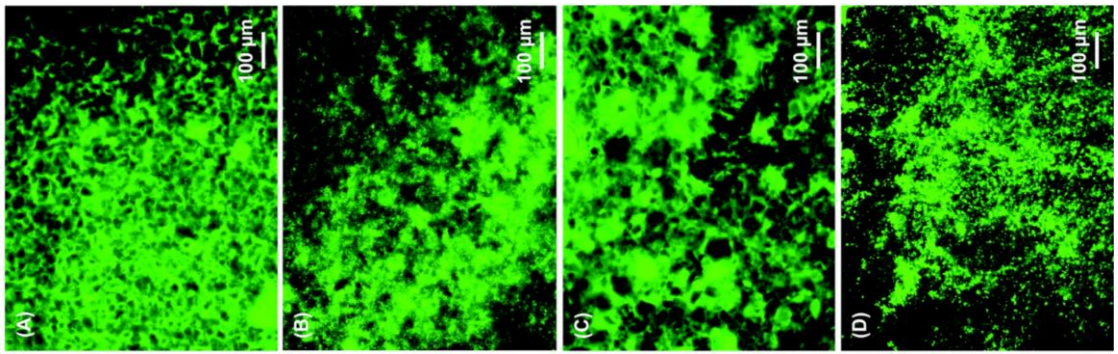
NEOGEN

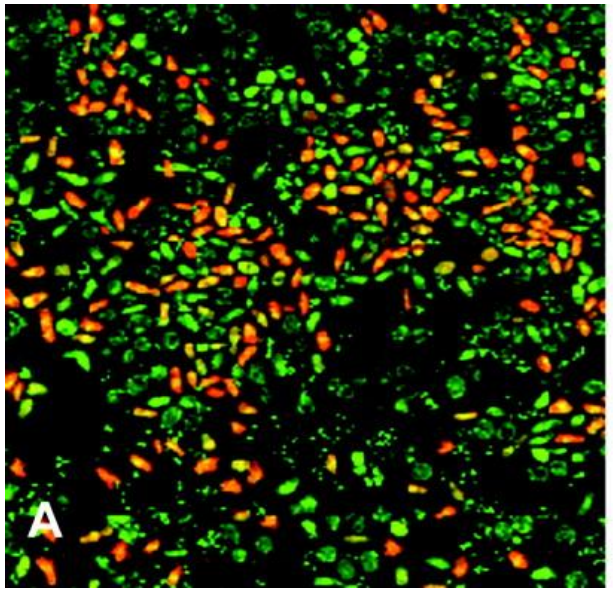
ANSR solutions – *Listeria* Right Now

Validation study – Neogen Corporation

- Tests conducted with *Listeria* spp alone and with background flora: *Enterococcus faecium, Bacillus subtilis* and *Pseudomonas aeruginosa*.







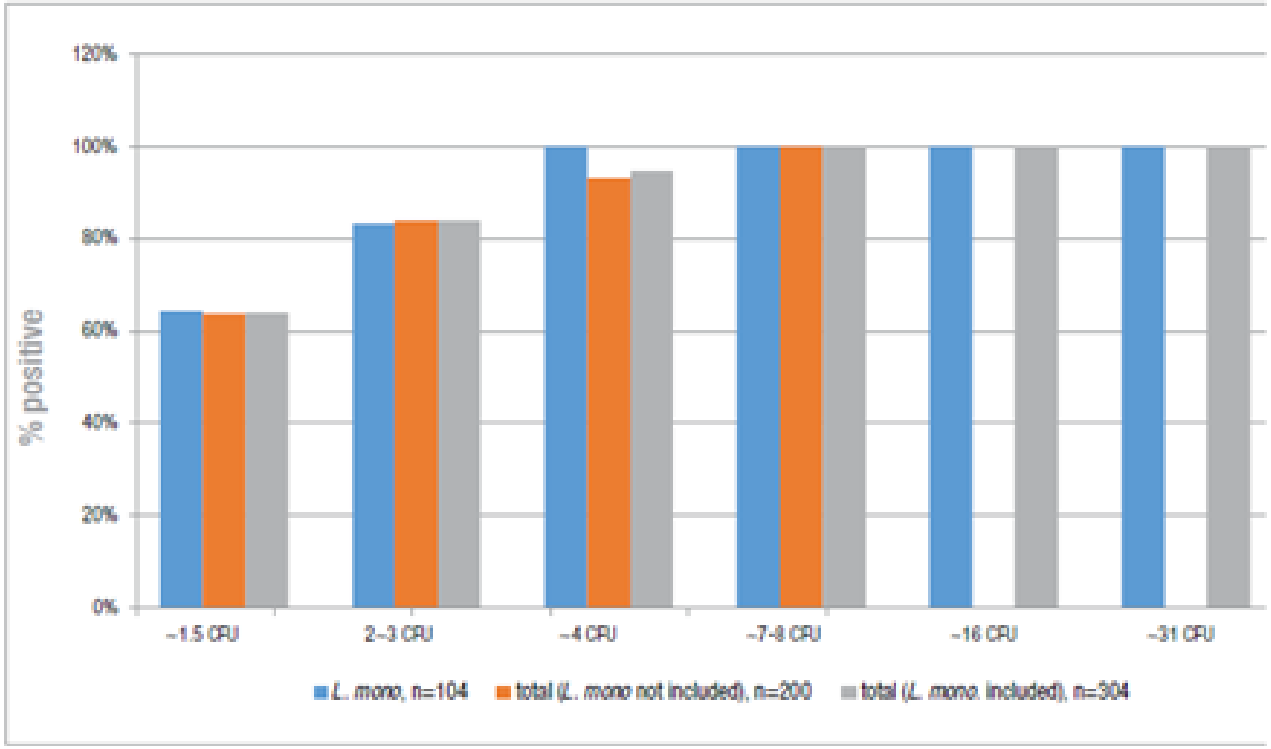
NEOGEN

ANSR solutions – *Listeria* Right Now

Validation study – Neogen Corporation

- **Results:**

- LOD of ~4 CFU / surface



Method: Inoculated directly onto swab

NEOGEN

Listeria Solutions

WHAT IF YOU COULD HAVE IT ALL?

GREATER ACCURACY

FASTER TIME TO RESULTS

MINIMAL INVESTMENT

BY AFNOR CERTIFICATION
NF VALIDATION
EN ISO 16140

NEO 35/03-01/16
ALTERNATIVE ANALYTICAL METHODS
FOR AGRIBUSINESS
http://nf-validation.afnor.org/en

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THANK YOU FOR YOUR ATTENTION

QUESTIONS?


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