Listeria monocytogenes Recalls are Up: Trends in Detection & Control

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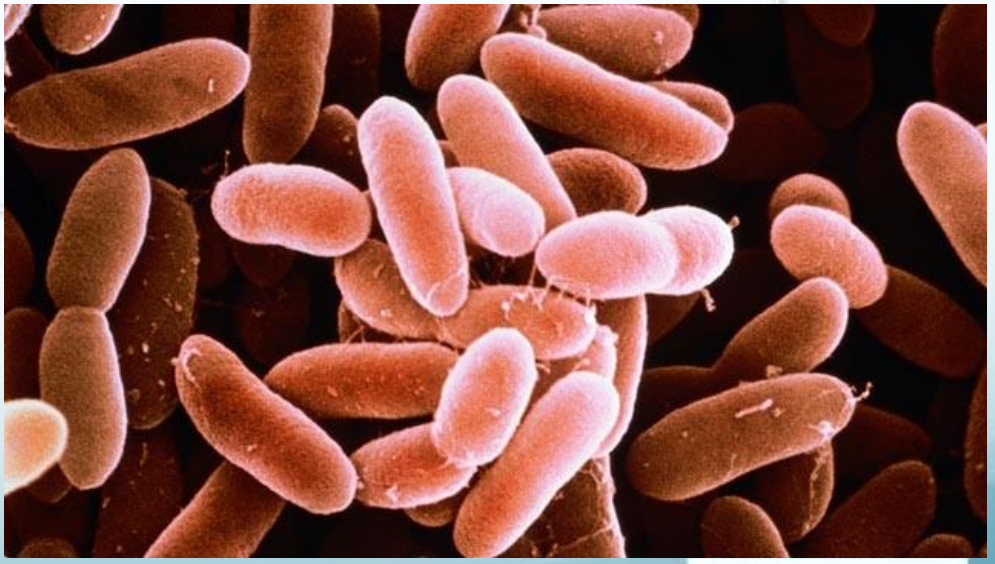
Outline

- Description of *Listeria monocytogenes*
- Prevalence in foods
- Key outbreaks/recalls
- Sources and risk analysis
- Control of *Listeria*
  - Chemical
  - Biological
  - Physical
- *Listeria* policy
- Diagnostics



History of *Listeria monocytogenes*

- Isolated from diseased rabbit in 1926
- Named after Lord Lister
- Animal Diseases

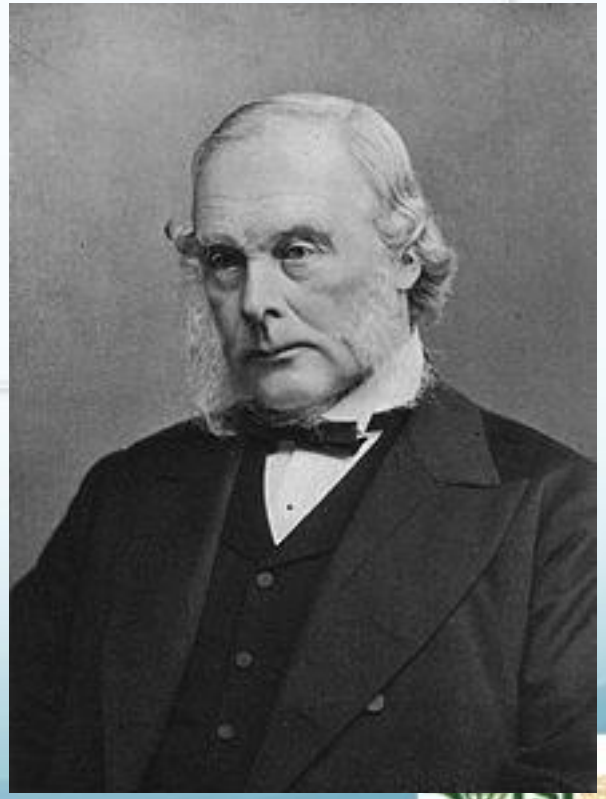
Circling disease

Silage sickness

Leukocytosis

Cheese sickness

Tiger river disease.



Human Listeriosis

Zoonotic

- Widely distributed in the environment
- Commonly linked to wild and domestic animals
- Asymptomatic carriers (10% carriers in the GI tract)

Foodborne

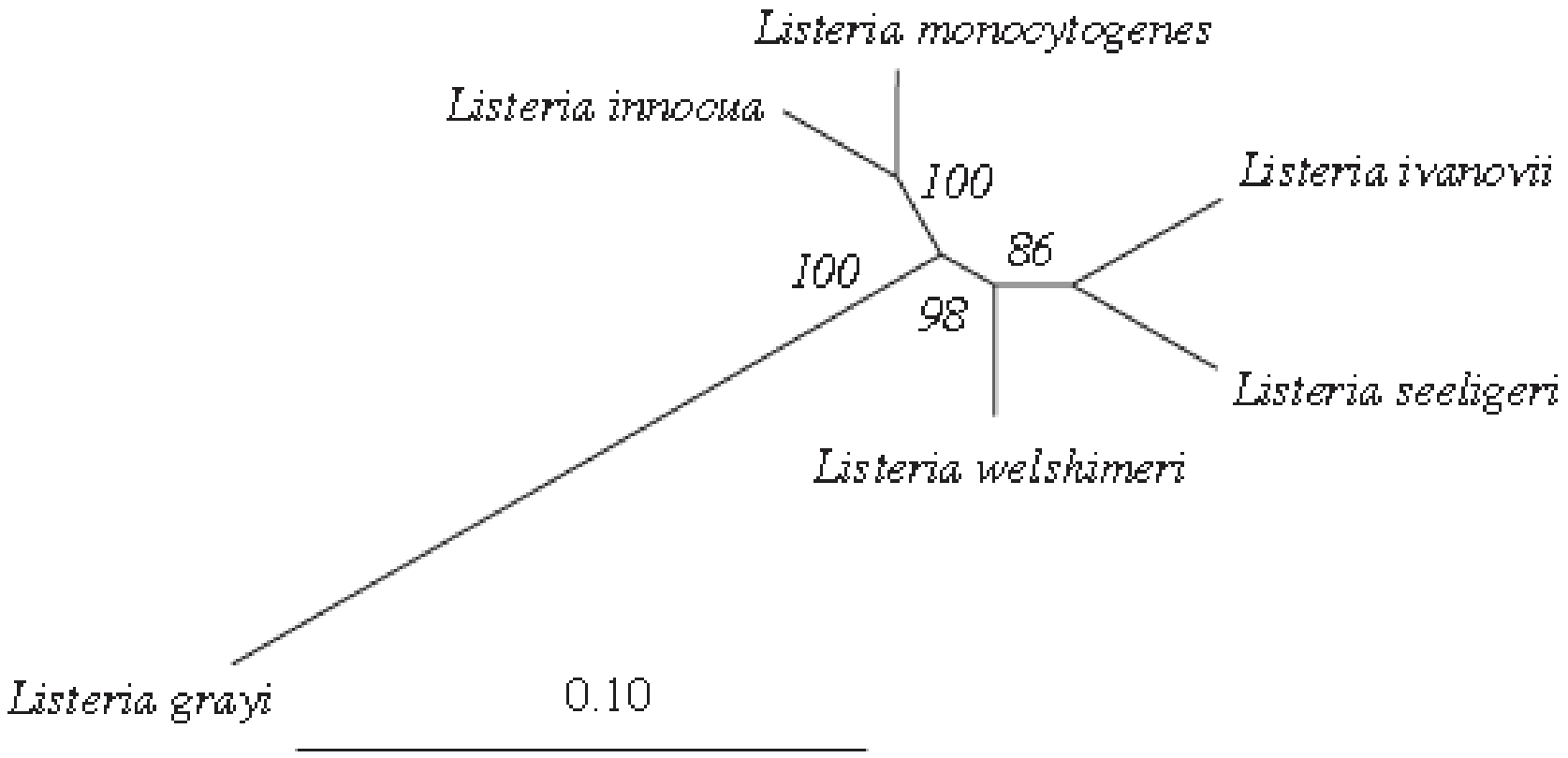
- More common in urban rather than rural populations.
- Linked to raw milk derived from cows suffering listeriosis.

Listeria

- *L. ivanovii*- pathogenic
- *L. welshimeri*
- *L. seeligeri*
- *L. grayi*
- *L. innocua*
- *L. monocytogenes*- pathogenic

- *Listeria floridensis*
- *Listeria aquatic*
- *Listeria cornellensis*
- *Listeria riparia*
- *Listeria grandensis*

Non-pathogenic


Listeria monocytogenes
Listeria innocua
100
Listeria ivanovii
86
100
98
Listeria seeligeri
Listeria welshimeri
Listeria grayi
0.10

Listeria monocytogenes

- 13serotypes

1/2a

1/2b

Implicated in foodborne listerisos in EU

1/2c

3a

3b

3c

4a

4b (Scott A)

Implicated in foodborne listeriosis North America

4c

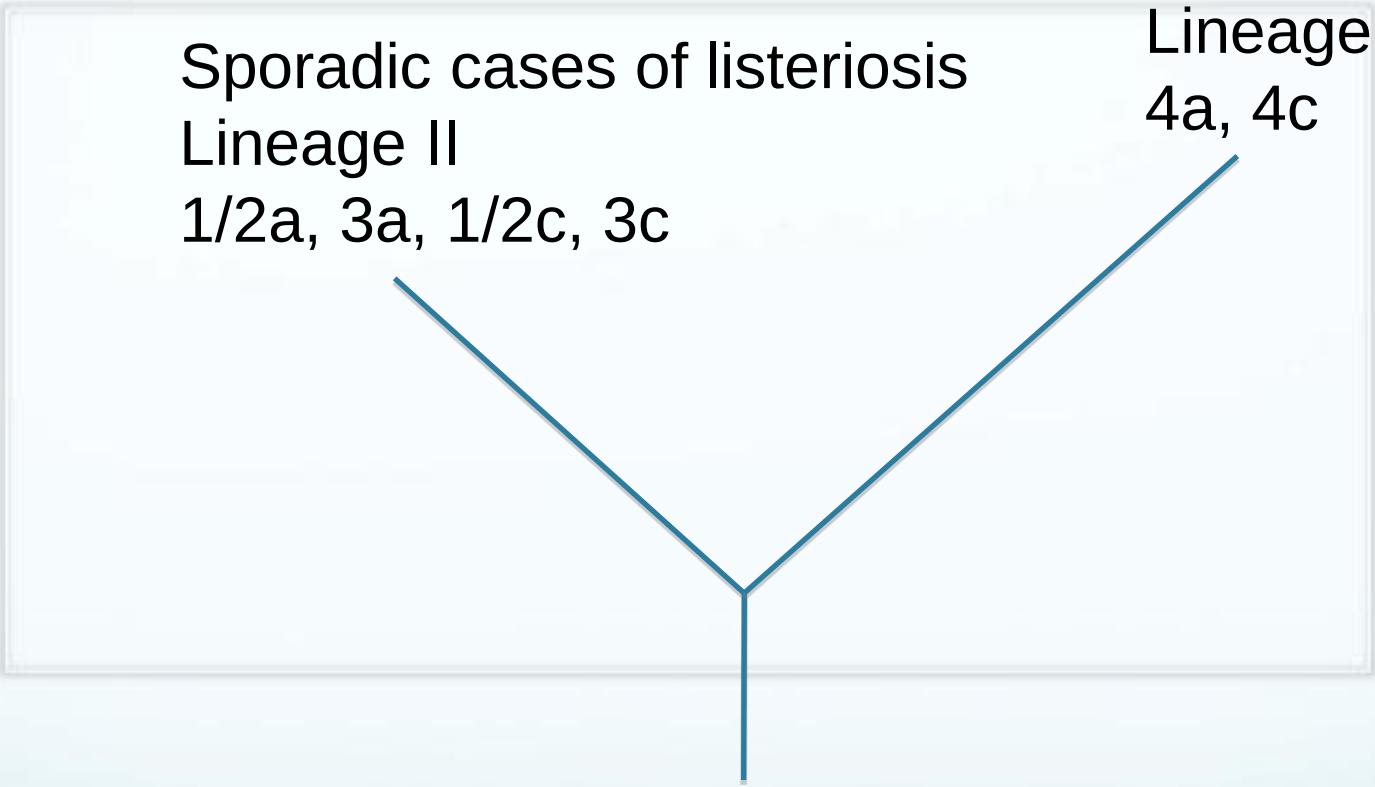
4d

4e

4ab

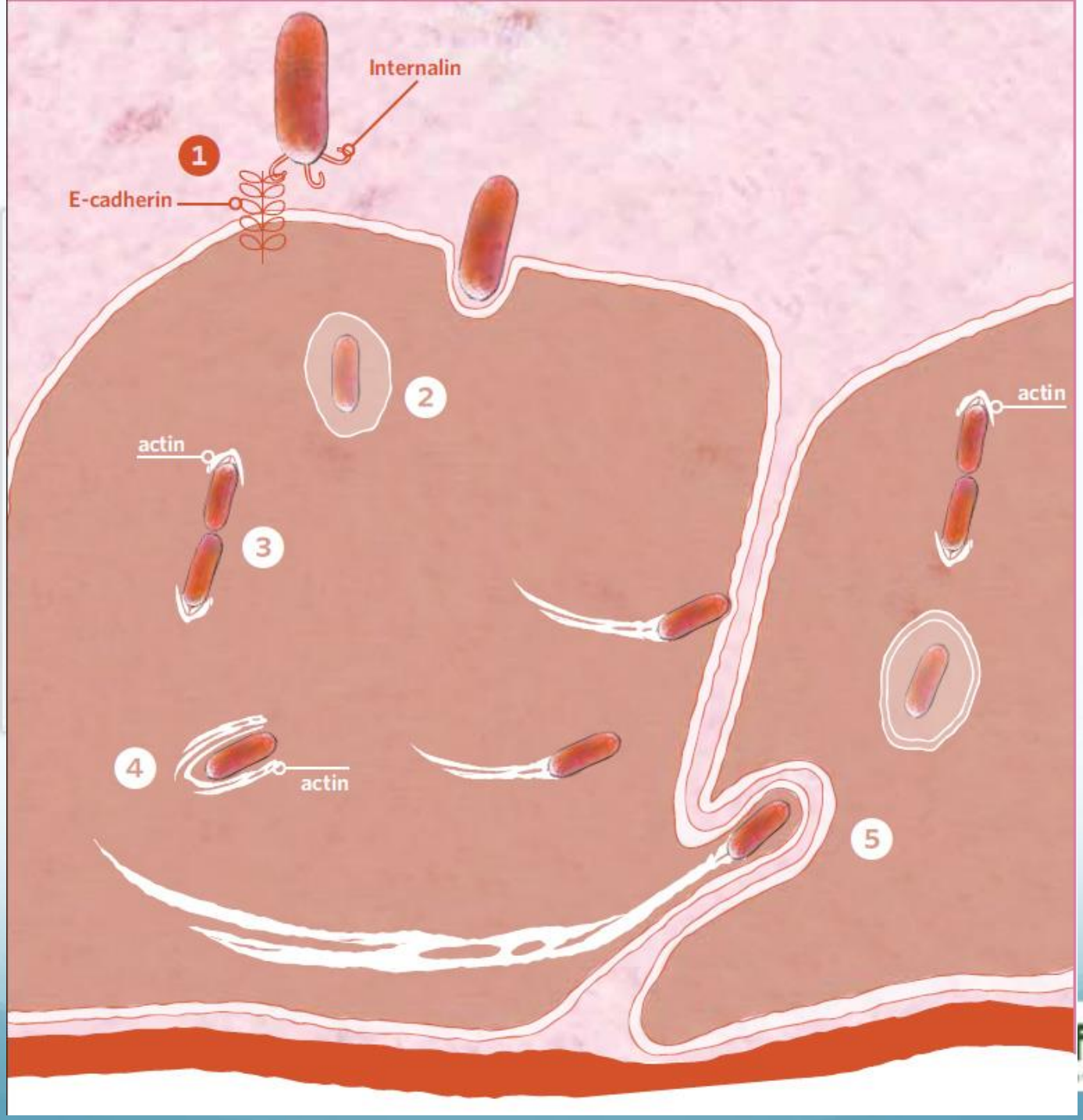
7

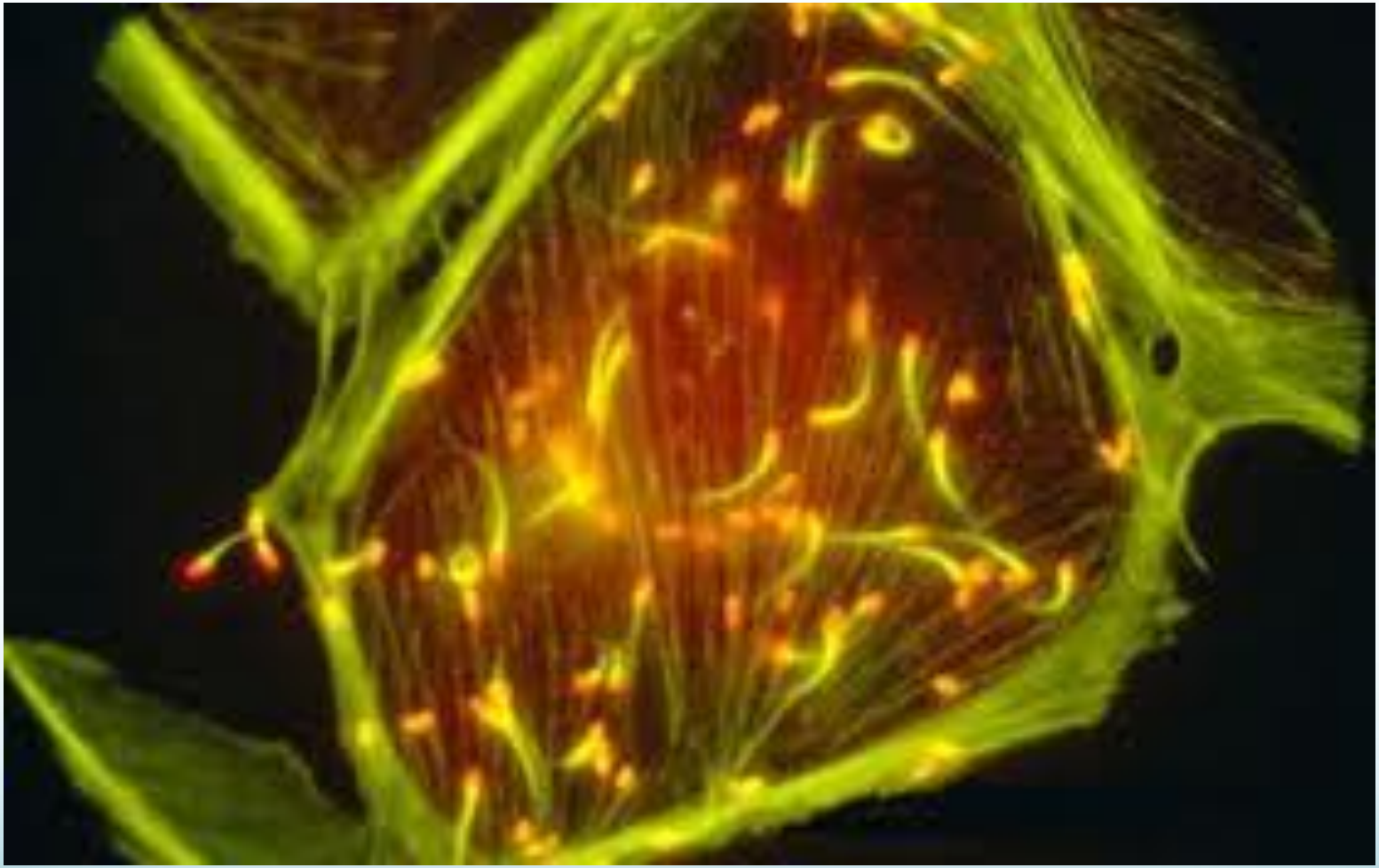
Genetic Lineage

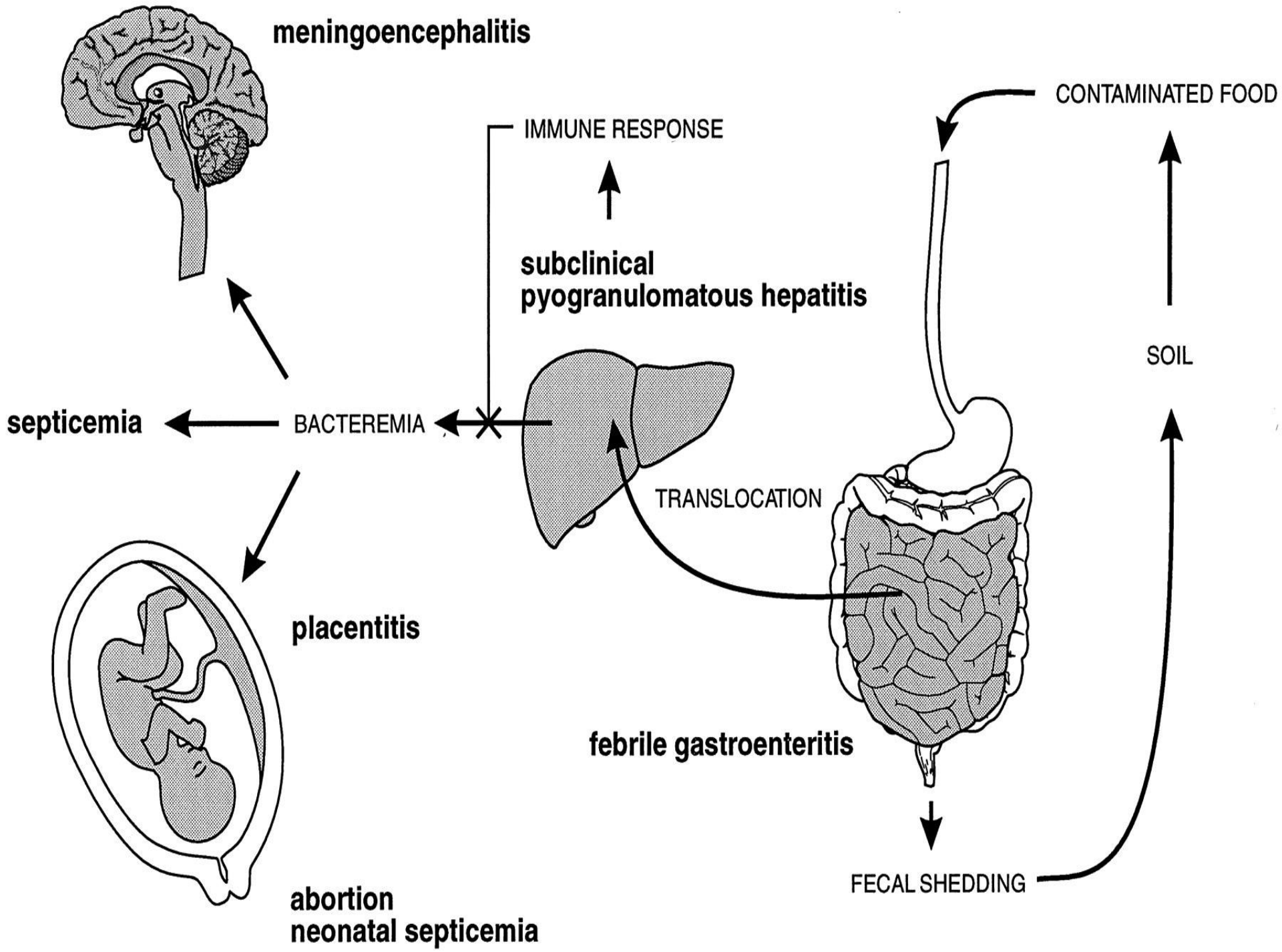


Lineage I
1/2b, 3b, 4b, 4d, 4e

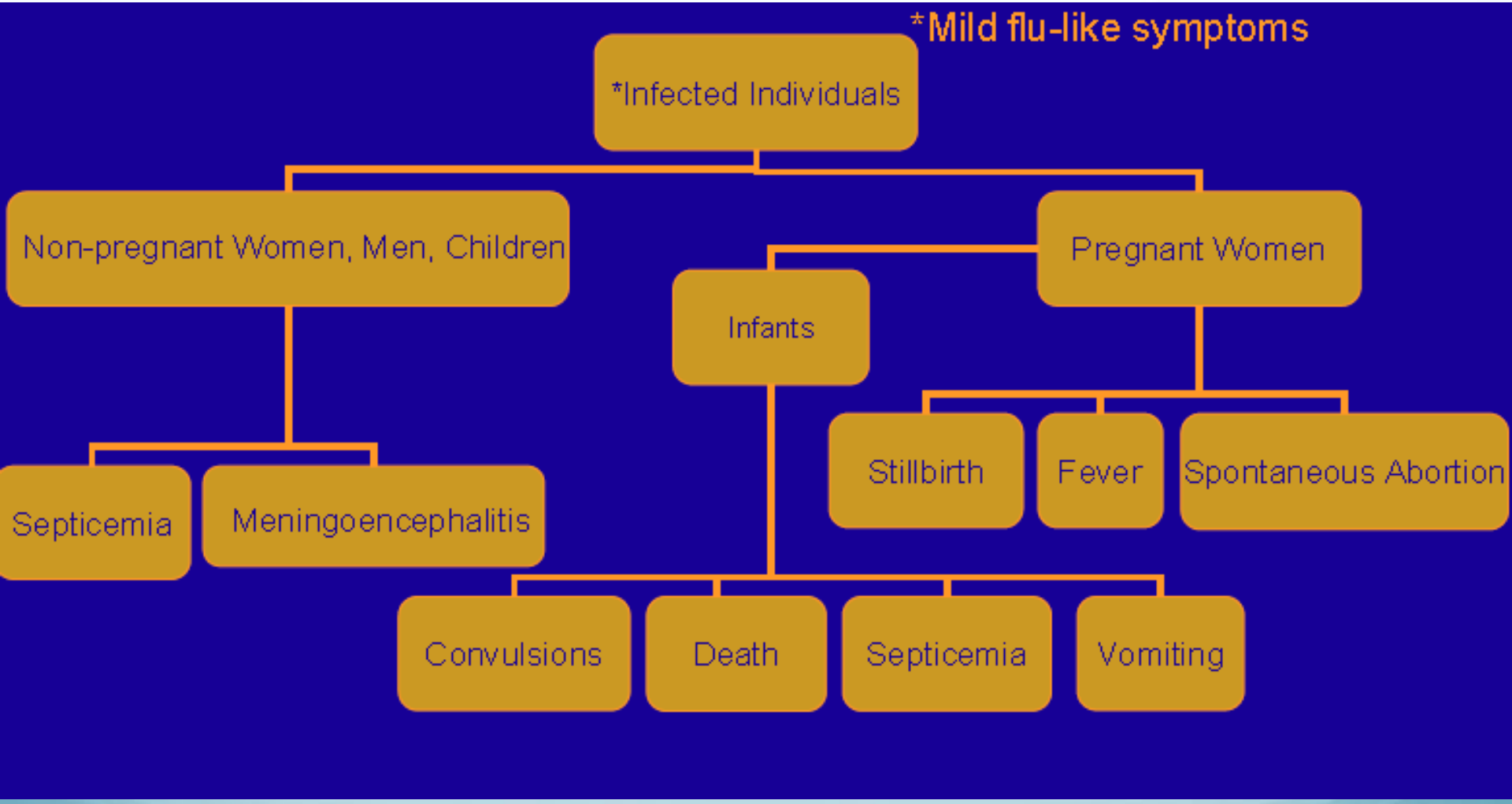
Majority of foodborne listeriosis

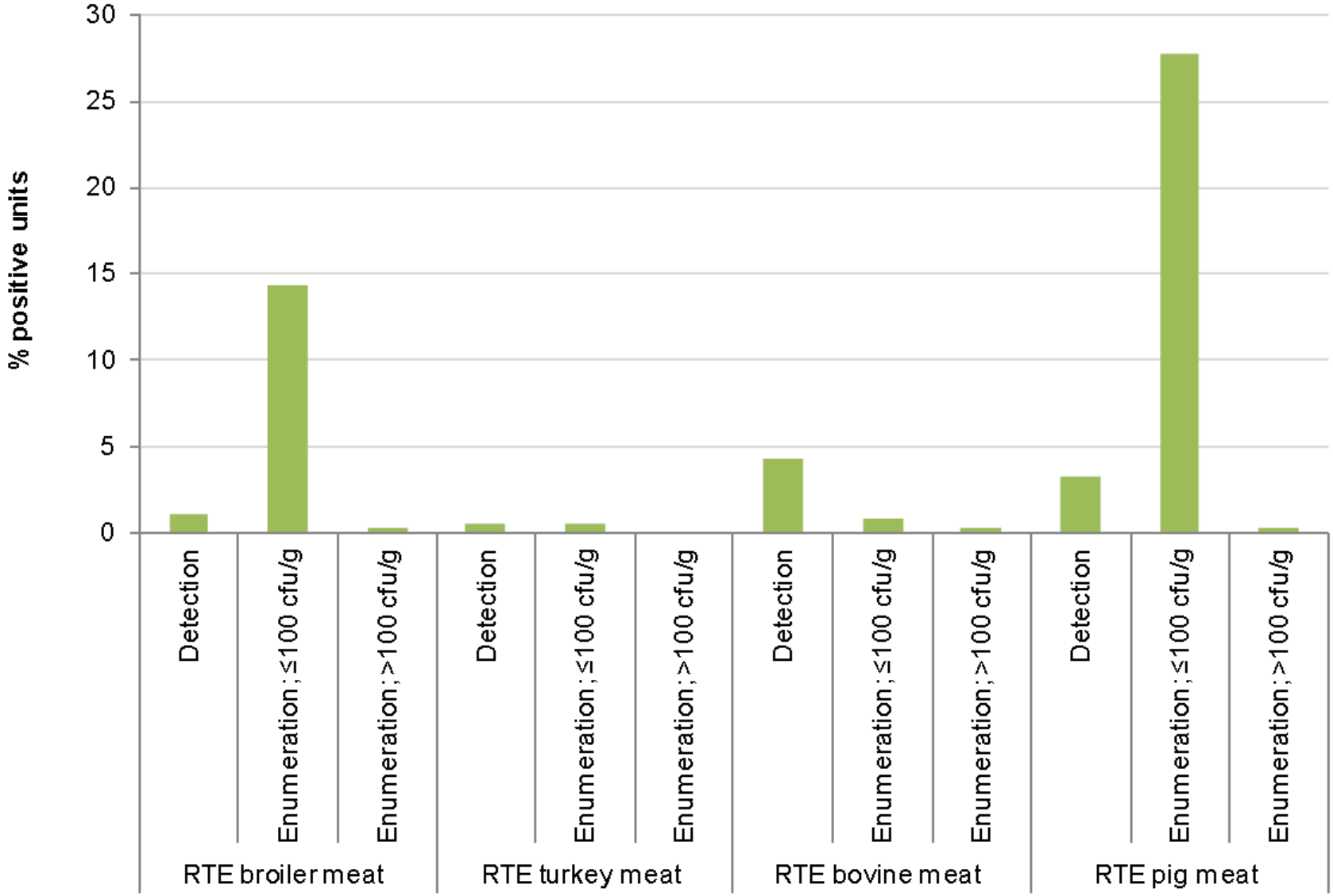

Internalin
1
E-cadherin
2
actin
3
actin
4
actin
5

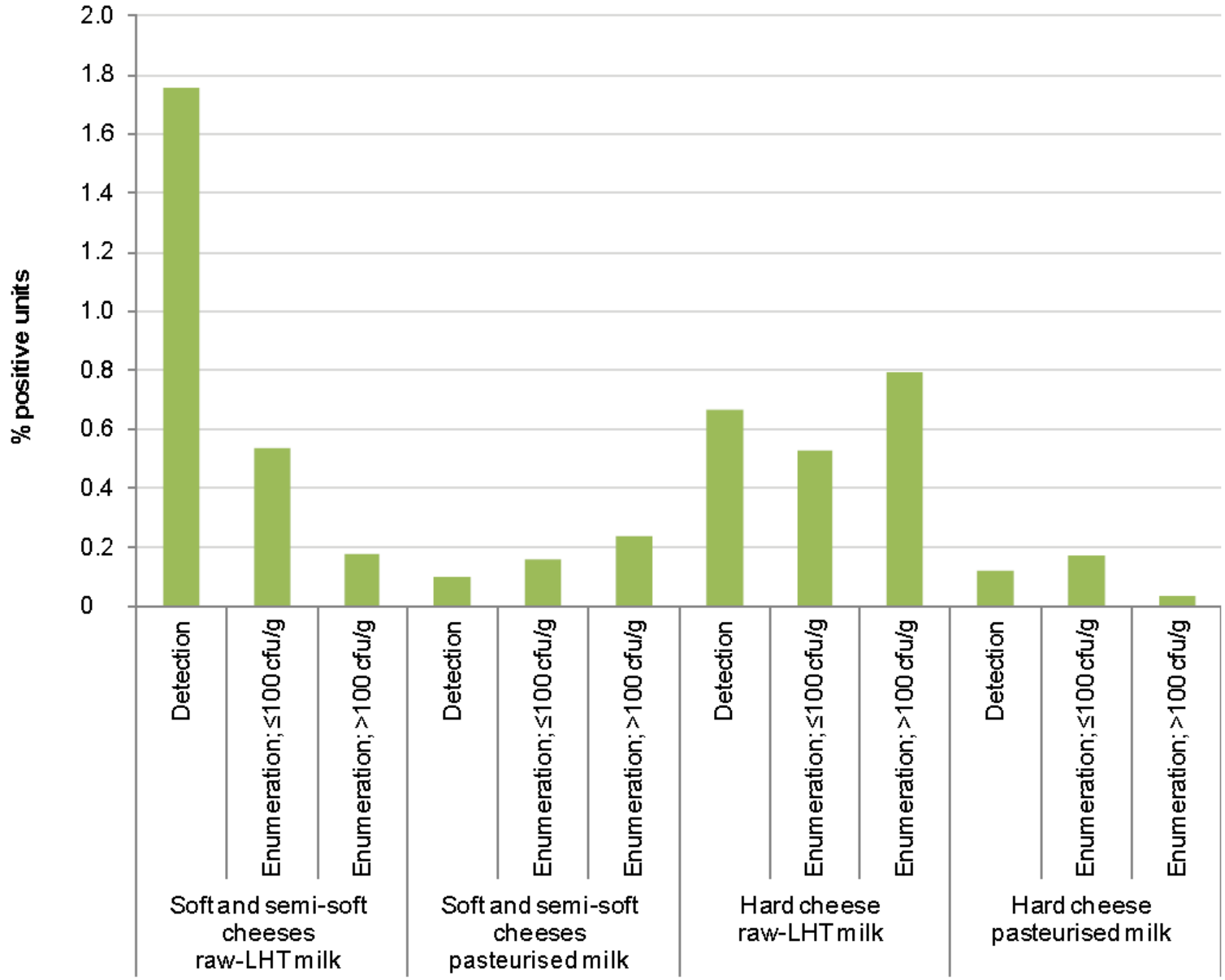


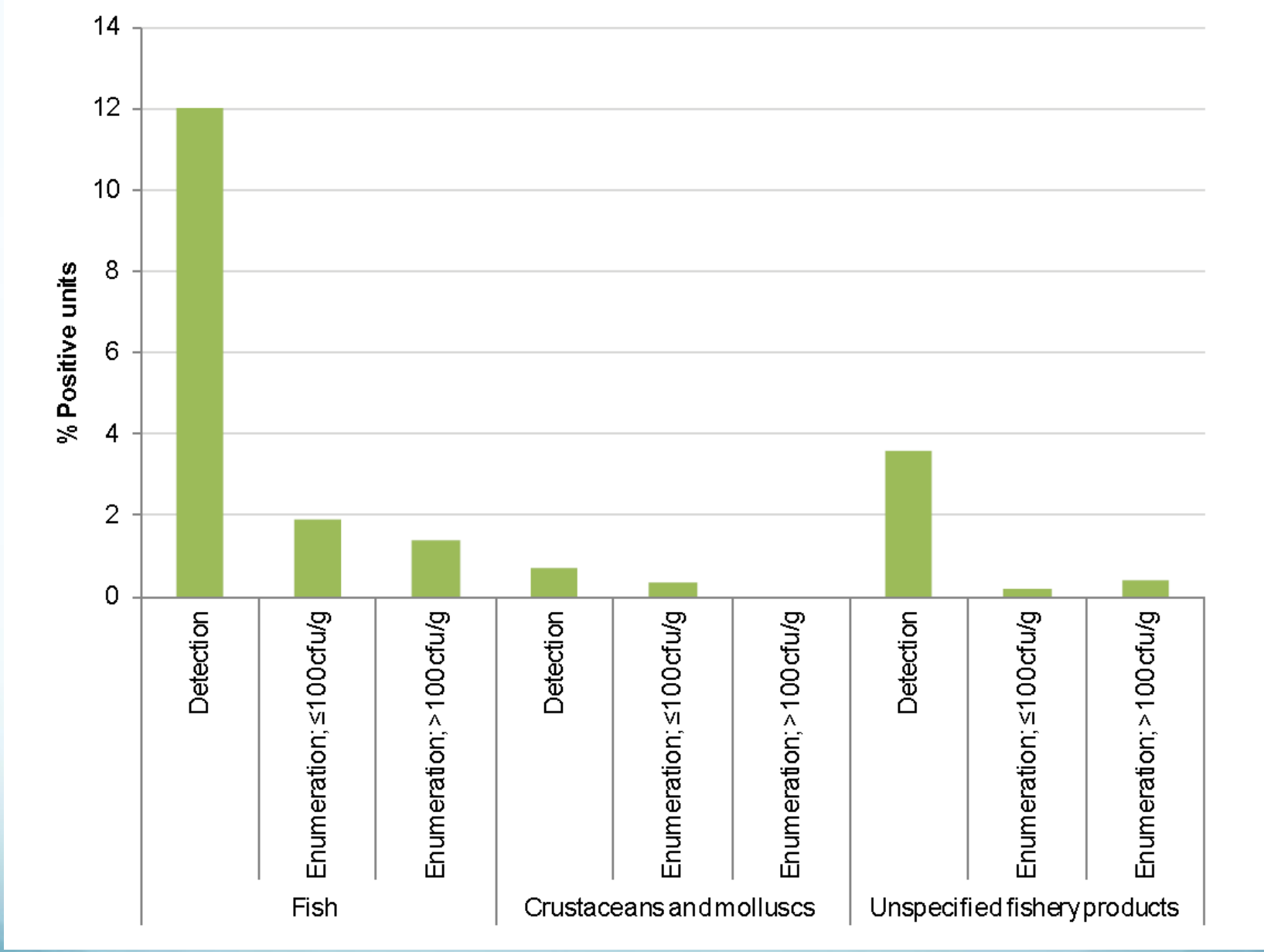

meningoencephalitis
CONTAMINATED FOOD
IMMUNE RESPONSE
subclinical
pyogranulomatous hepatitis
SOIL
septicemia
BACTEREMIA
TRANSLOCATION
placentitis
febrile gastroenteritis
FECAL SHEDDING
abortion
neonatal septicemia

Listeria Infection




% positive units
30
25
20
15
10
5
0
Detection
Enumeration; ≤100 cfu/g
Enumeration; >100 cfu/g
RTE broiler meat
RTE turkey meat
RTE bovine meat
RTE pig meat


% positive units
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Detection
Enumeration; ≤100 cfu/g
Enumeration; >100 cfu/g
Soft and semi-soft cheeses raw-LHT milk
Soft and semi-soft cheeses pasteurised milk
Hard cheese raw-LHT milk
Hard cheese pasteurised milk


% Positive units
14
12
10
8
6
4
2
0
Detection
Enumeration; ≤100cfu/g
Enumeration; > 100cfu/g
Detection
Enumeration; ≤100cfu/g
Enumeration; > 100cfu/g
Detection
Enumeration; ≤100cfu/g
Enumeration; > 100cfu/g
Fish
Crustaceans and molluscs
Unspecified fishery products

Key Products linked to LM (all RTE)

Seafood Salads (4.7%)

Smoked seafood (4.31%)

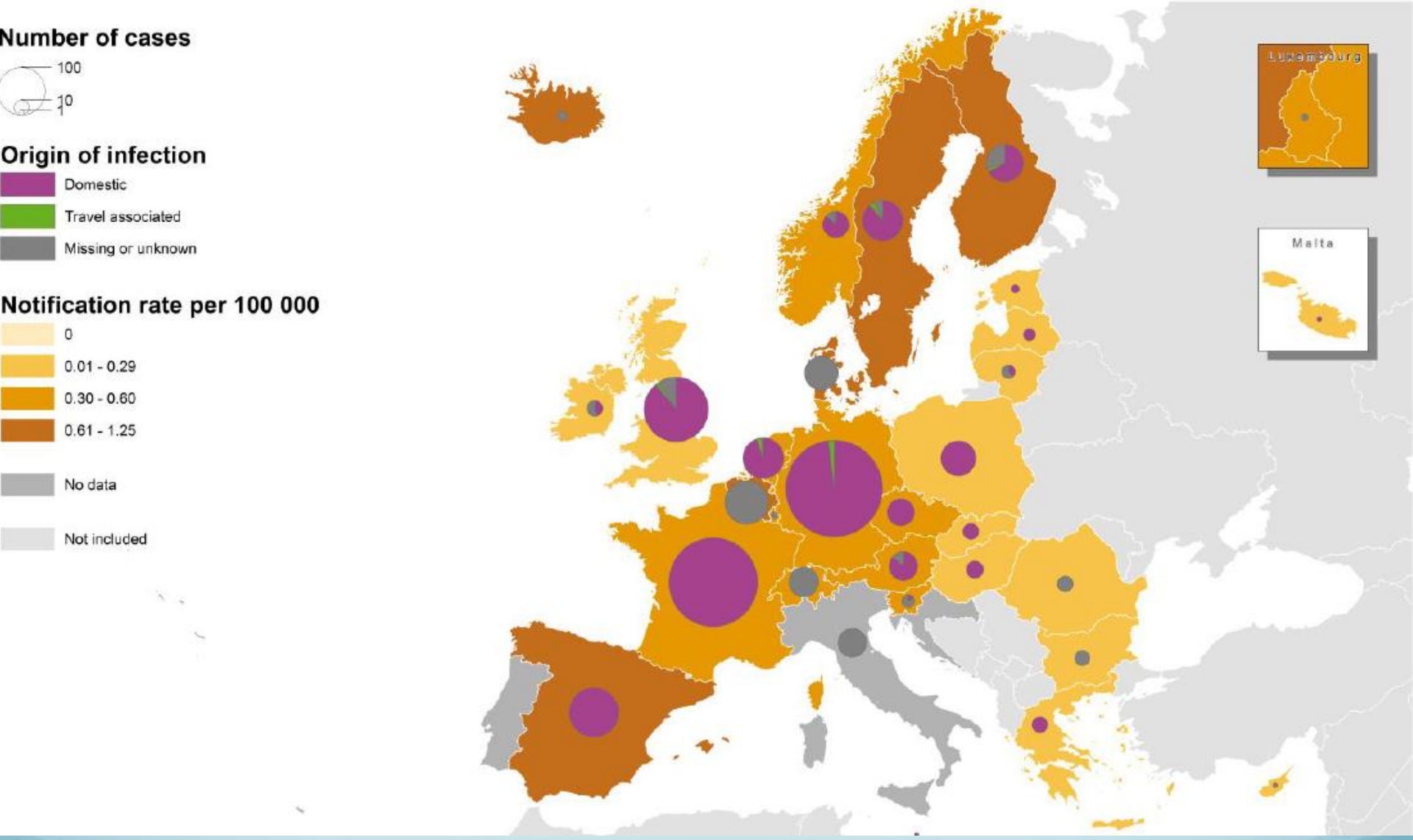
Deli salads (2.36%)

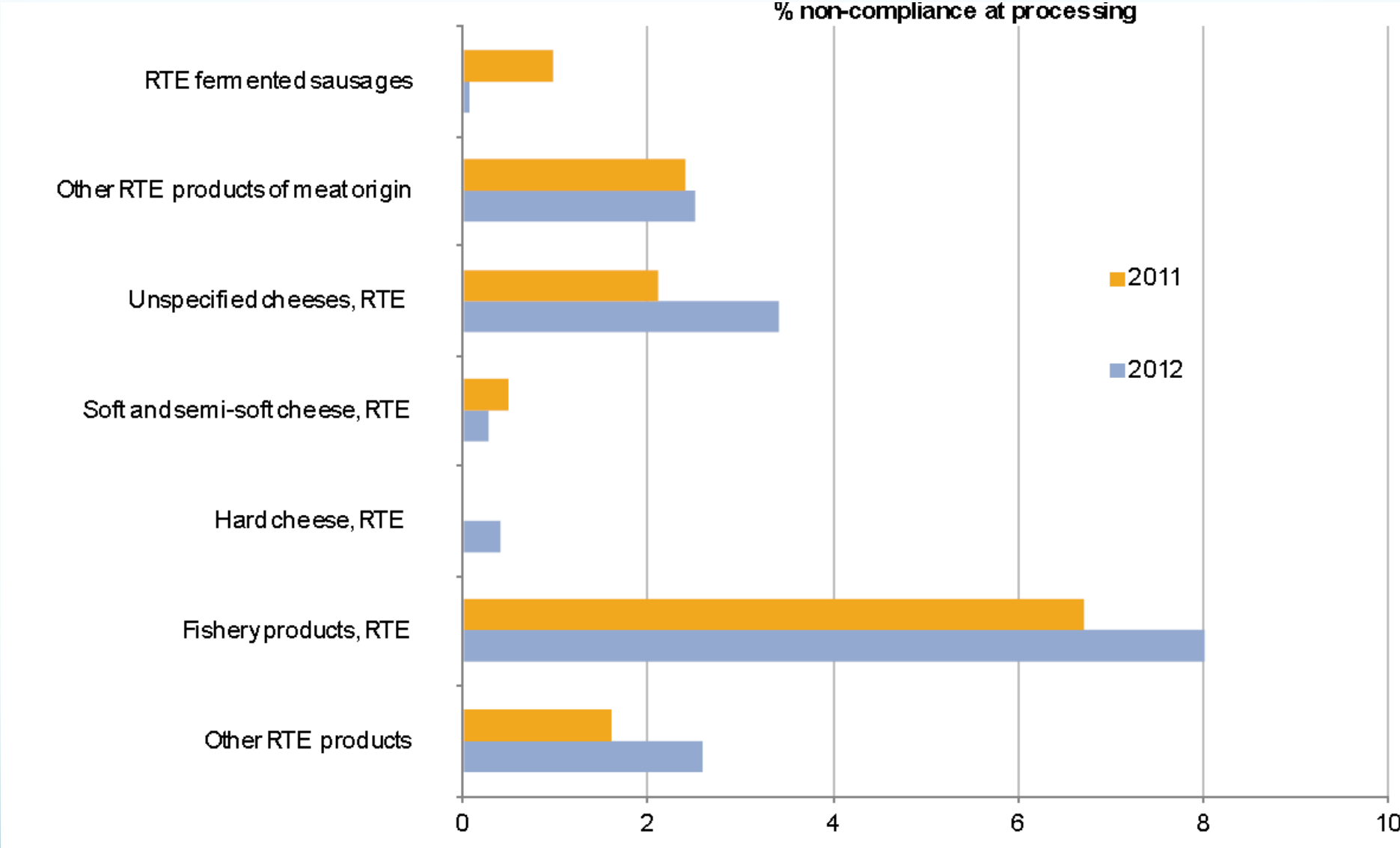
Deli meats (1.82%)

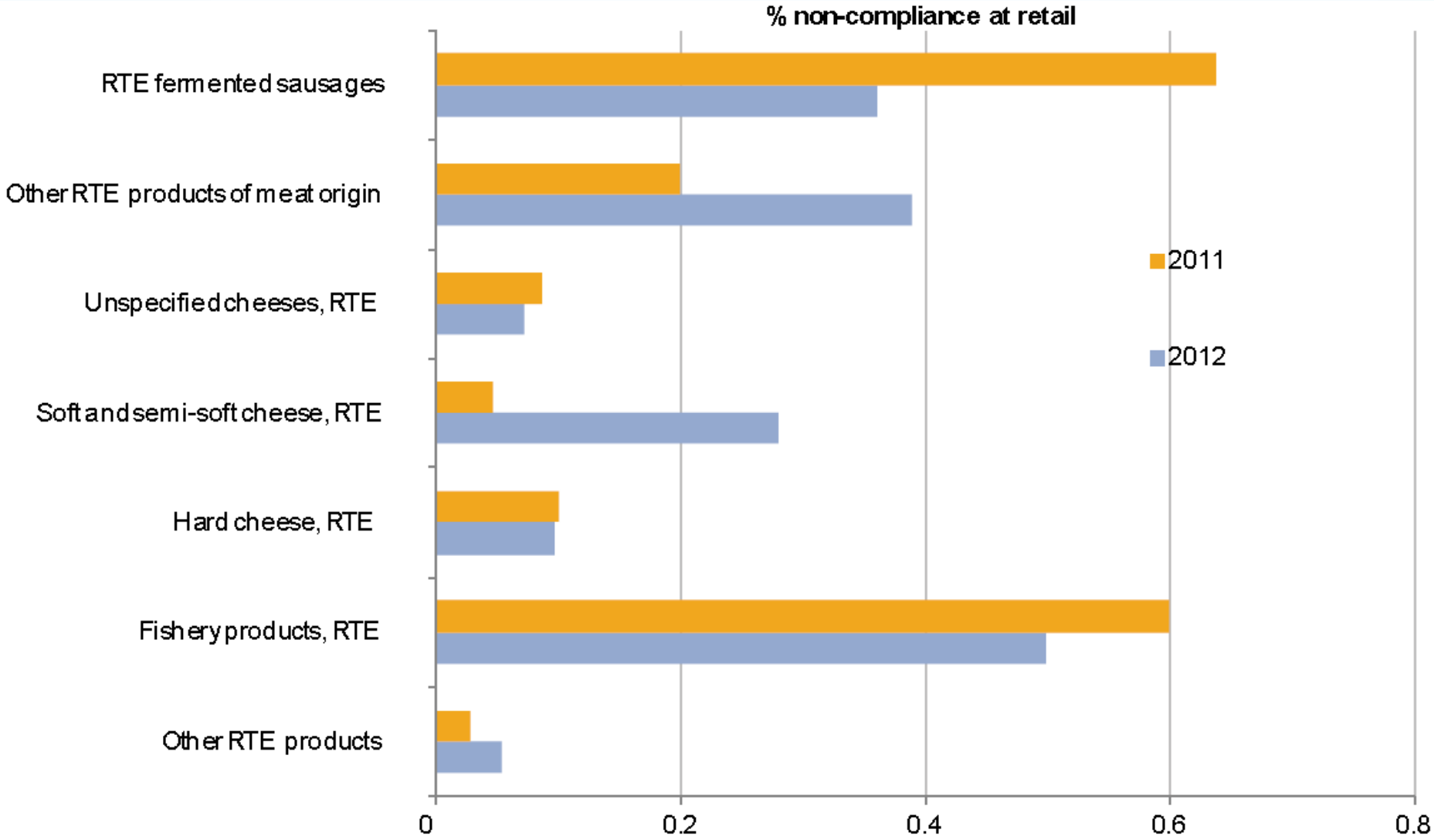
Luncheon meats (0.89%)

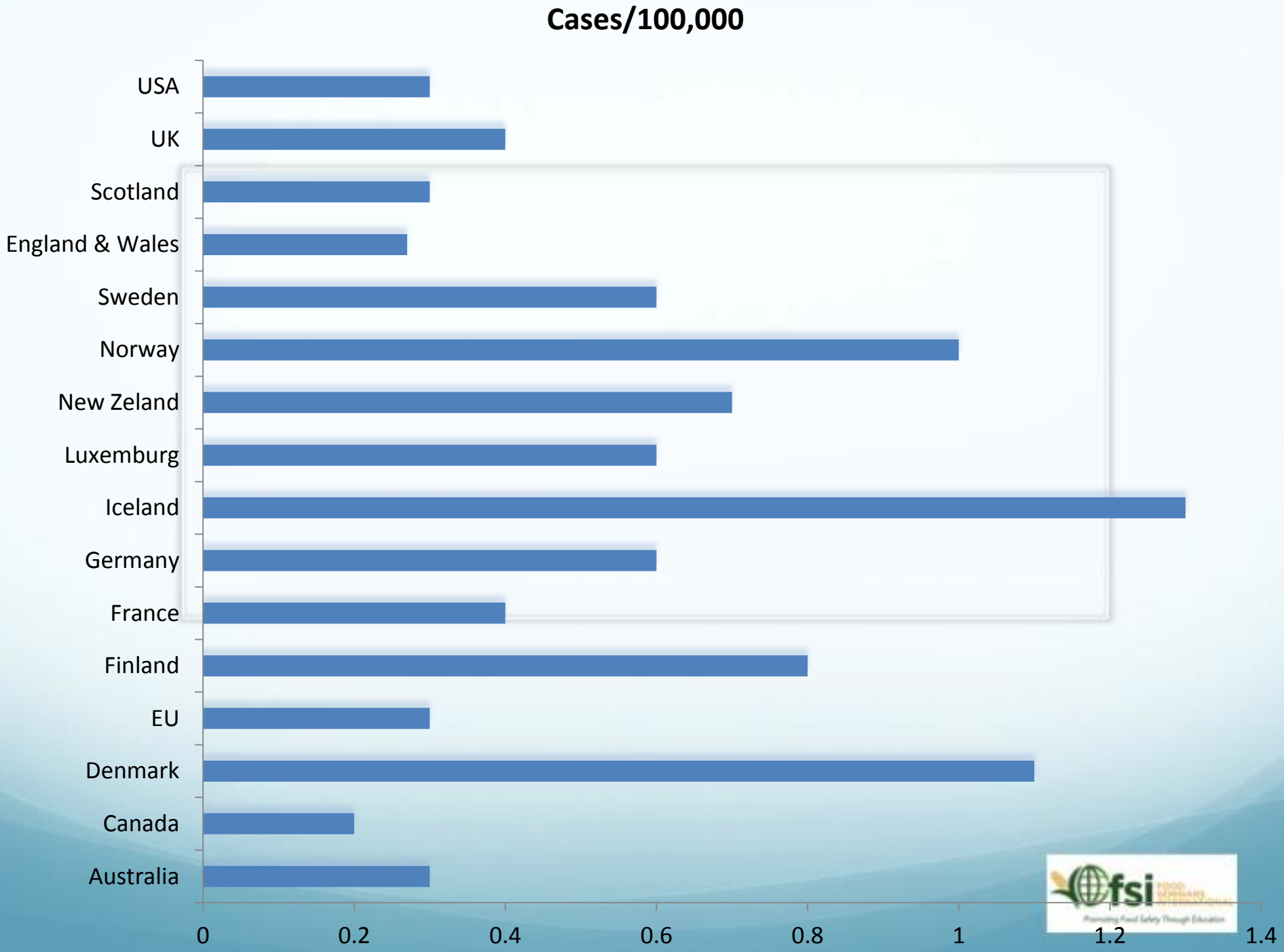
Soft cheese (0.46%)

(Gombas et al., 2003)

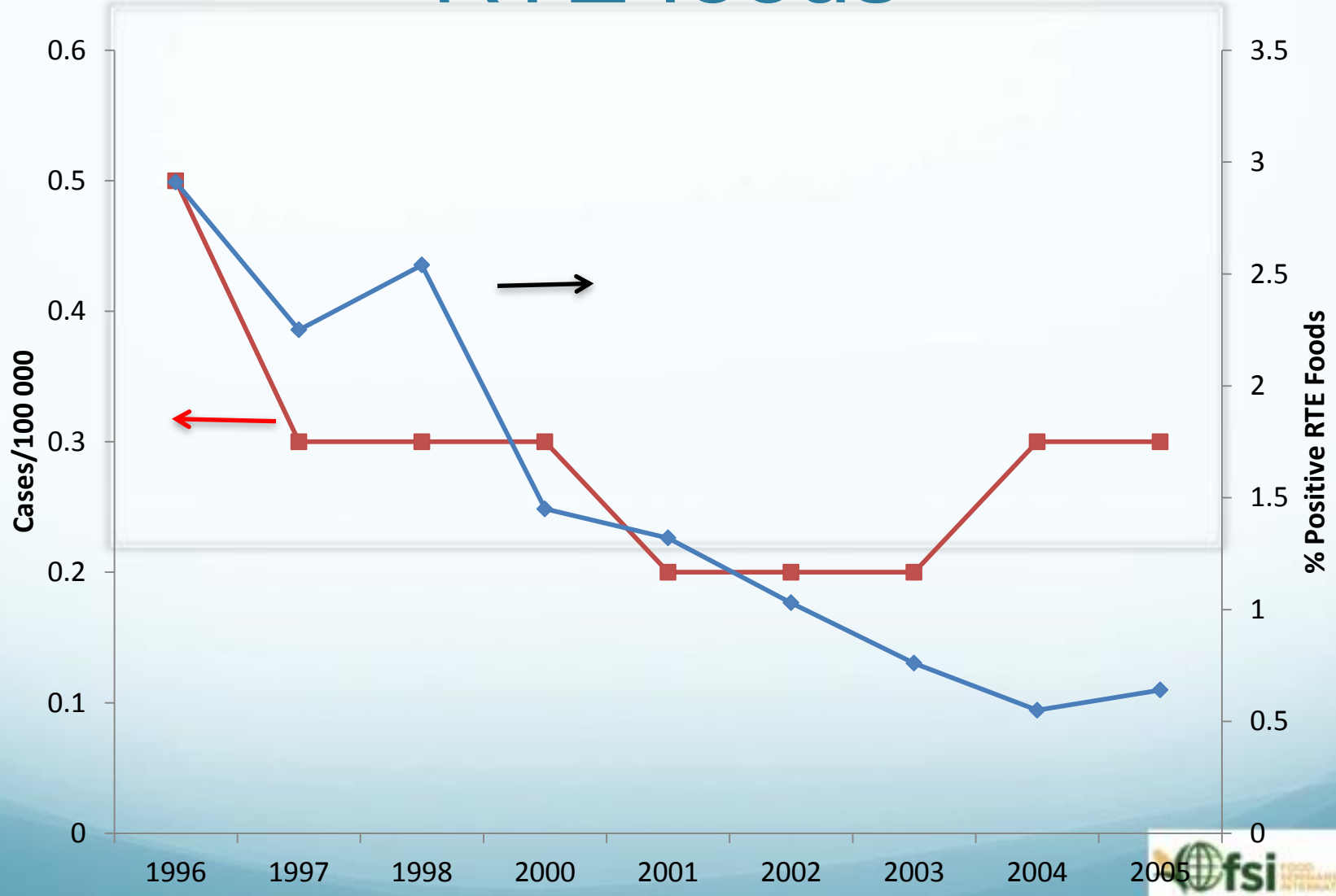

Number of cases
100
10
1
Origin of infection
Domestic
Travel associated
Missing or unknown
Notification rate per 100 000
0
0.01 - 0.29
0.30 - 0.60
0.61 - 1.25
No data
Not included
Luxembourg
Malta


% non-compliance at processing
RTE fermented sausages
Other RTE products of meat origin
Unspecified cheeses, RTE
Soft and semi-soft cheese, RTE
Hard cheese, RTE
Fishery products, RTE
Other RTE products
2011
2012
0
2
4
6
8
10


% non-compliance at retail
RTE fermented sausages
Other RTE products of meat origin
Unspecified cheeses, RTE
Soft and semi-soft cheese, RTE
Hard cheese, RTE
Fishery products, RTE
Other RTE products
2011
2012
0
0.2
0.4
0.6
0.8


Cases/100,000
USA
UK
Scotland
England & Wales
Sweden
Norway
New Zeland
Luxemburg
Iceland
Germany
France
Finland
EU
Denmark
Canada
Australia
0
0.2
0.4
0.6
0.8
1
1.2
1.4

Listeria cases and carriage on RTE foods



FOOD SAFETY PROGRESS REPORT FOR 2012

Disease Agents	Percentage change in 2012 compared with 2006–2008		2012 rate per 100,000 Population	2020 target rate per 100,000 Population	CDC estimates that...
Campylobacter		14% increase	14.30	8.5	For every *Campylobacter* case reported, there are 30 cases not diagnosed
Escherichia coli O157		No change	1.12	0.6	For every *E. coli* O157 case reported, there are 26 cases not diagnosed
Listeria		No change	0.25	0.2	For every *Listeria* case reported, there are 2 cases not diagnosed
Salmonella		No change	16.42	11.4	For every *Salmonella* case reported, there are 29 cases not diagnosed
Vibrio		43% increase	0.41	0.2	For every *Vibrio parahaemolyticus* case reported, there are 142 cases not diagnosed
Yersinia		No change	0.33	0.3	For every *Yersinia* case reported, there are 123 cases not diagnosed

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

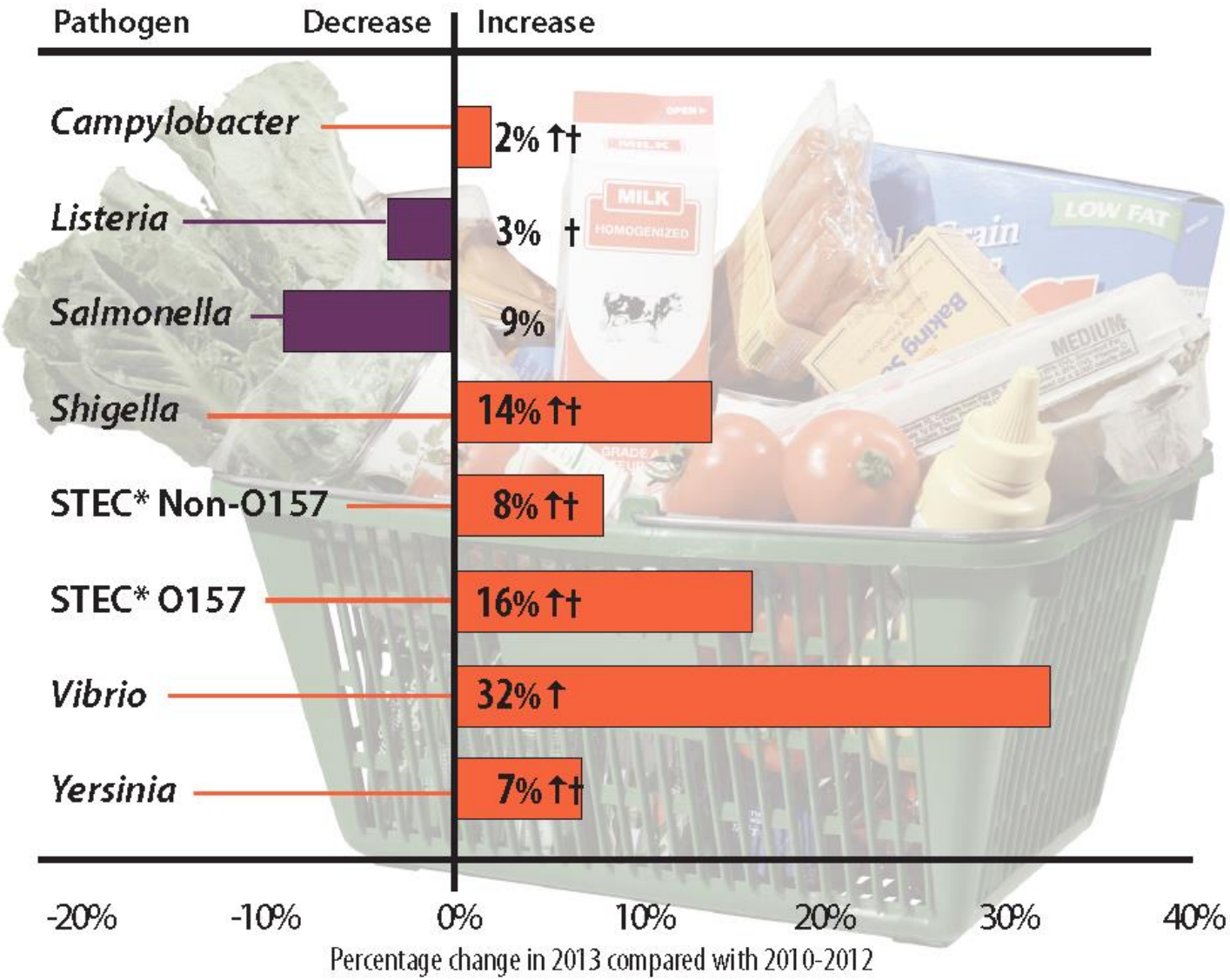
For more information, see http://www.cdc.gov/foodnet/

Preliminary FoodNet 2012 Data

April 2013

CS239349

Changes in incidence of laboratory-confirmed bacterial infections, US, 2013



* Shiga toxin-producing *Escherichia coli* † Not statistically significant www.cdc.gov/foodnet/ April 2014

Significant Outbreaks

Year	Product	Number of cases	Deaths
1981 Canada	Coleslaw	41	5
1985 USA	Mexican Cheese	142	48
1992 France	Deli Meat	279	85
2004-2007 USA	Queso Fresco	135	22
2008 Canada	Deli Meats	65	20

Listeria Initiative

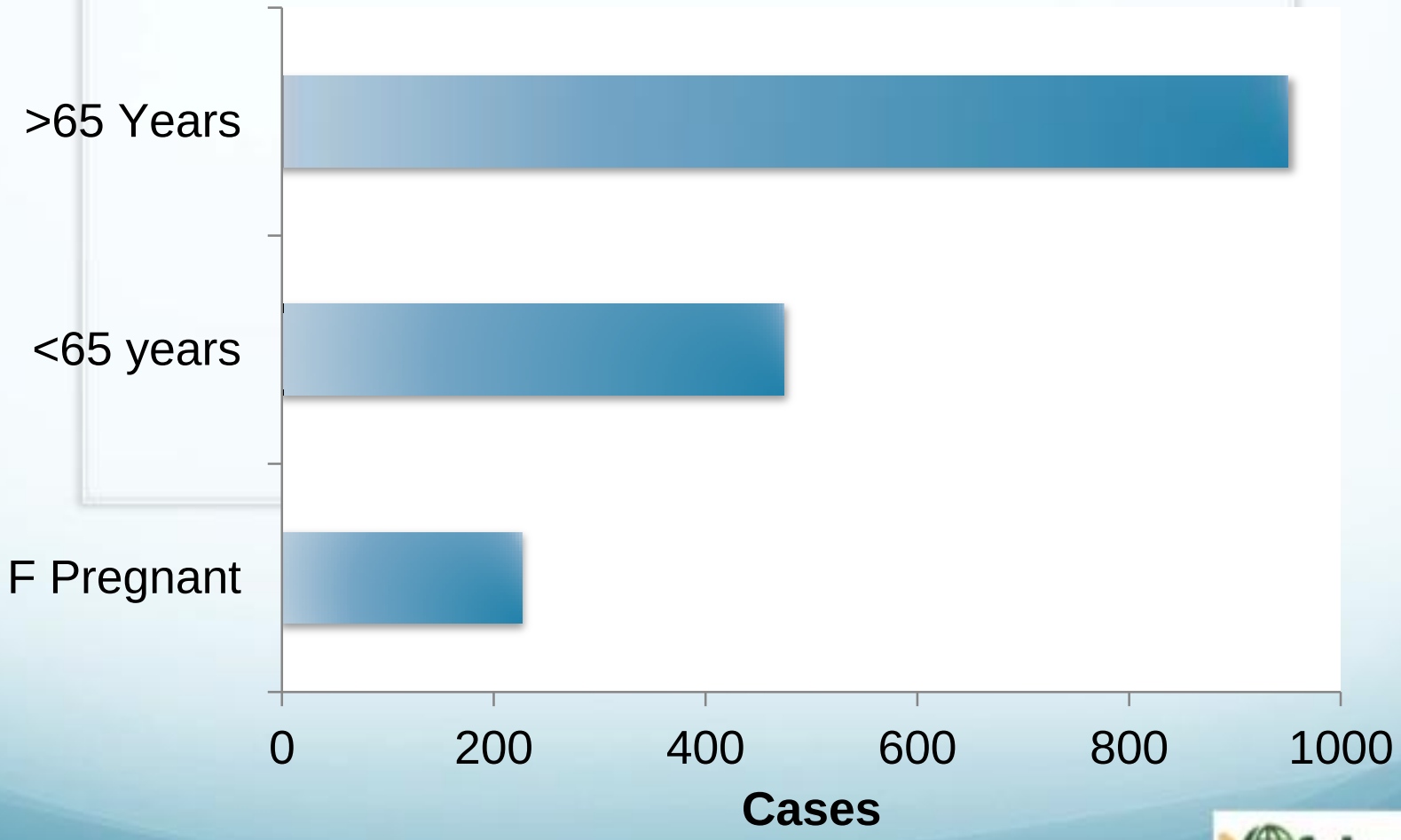
- CDC FoodNet
- *Listeria* cases and outbreaks US 2009-2011
- 1651 cases
- 21% fatality rate
- 2.4 Outbreaks per year
- 38% decline since 2003

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION™

Recent Outbreaks

Year	Cases	Implicated Food
2010	14 (2 deaths)	Hog Head Cheese
2010	4	Hospital (source unknown)
2010	10 (5 deaths)	Pre-cut Celery
2010	6	Mexican Style Cheese
2011	2	Chive Cheese
2011	147 (33 deaths)	Cantaloupe
2011	15 (3 deaths)	Blue-Veined Cheese
2012	11	Camembert Cheese
2013	5 (1 death)	Soft Cheese
2014	8 (1 death)	Mexican Style Cheese

Listeria Cases US 2009-2011



Listeria Recalls 2014

Country	Product	Month
US	Curry Chicken Salad	April
US	Yobaby Yogurt Cups	April
UK	Airline Dinners	April
US	Italian Salad	March
US	Cheese Spread	March
US	Peanut Butter	March
Canada	Mozzarella Cheese	March
US	Ice Cream	March
Canada	Caser Salad	Feb
US	Hummus and Wraps	Feb
Canada	Od Cheddar Cheese	Feb
Canada	Smoked Salmon	Jan
US	Raw Milk	Jan
Australia	Cheese	Jan

Listeria Outbreak linked to Cantaloupe

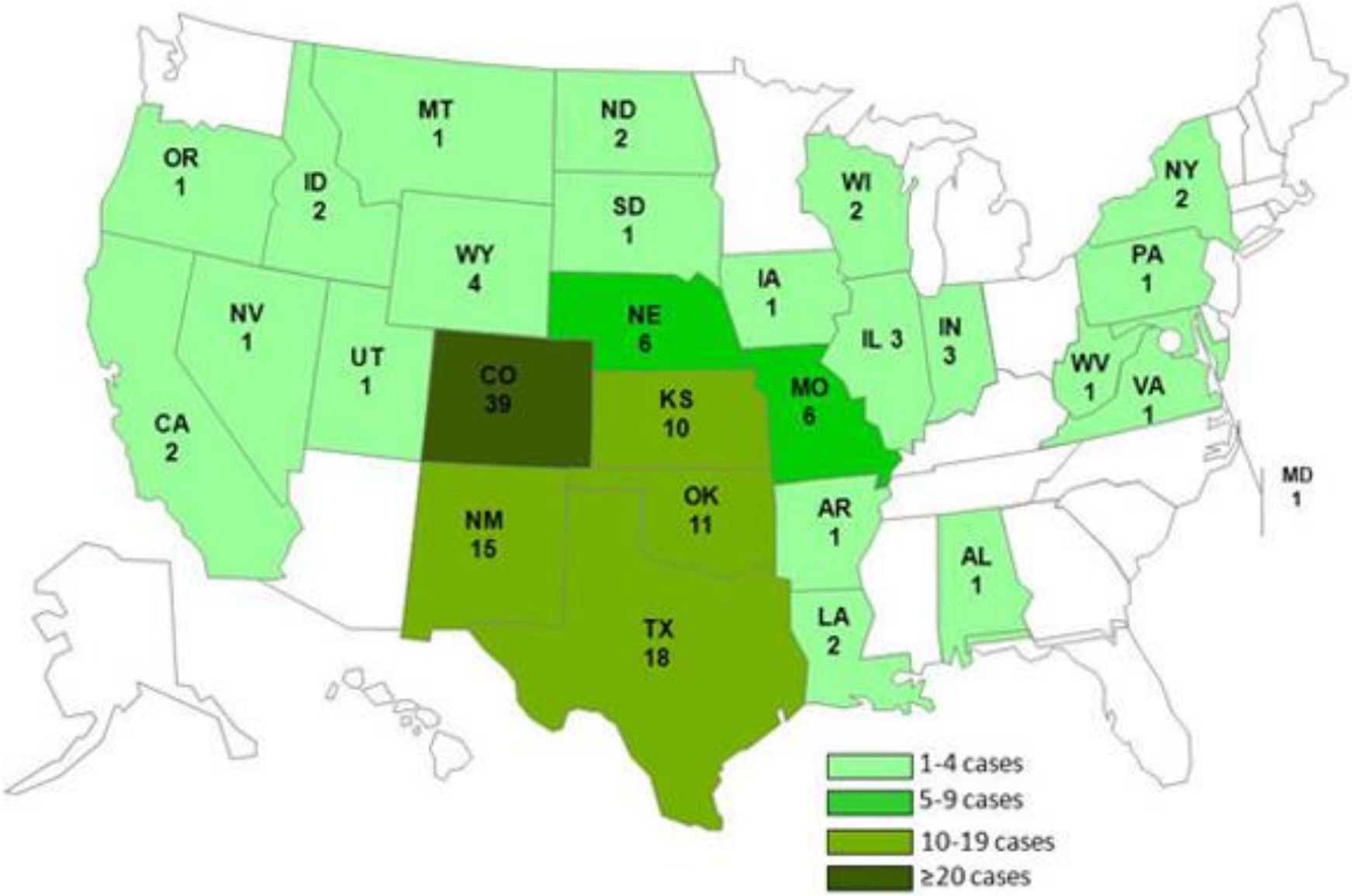
- 20 years old buisness
- Family run farm (4 generations)
- Tourist attraction

- Major producer within Colarado

Outbreak

- Product recall Sept 14th : From July 29th
- 300, 000 cartons
- Positive *Listeria monocytogenes*: Cantaloupe and Facility environment
- 4 strains of *L. monocytogenes* identified (serotype 1/2a and 1/2b)

- 160 cases
- 30 deaths (one miscarriage)
- 24 States
- Age range: 35-96 years Median 78 years
- 58% female
- All melons from Colorado removed from shelfs ($8m loss; estimated $150m losses)

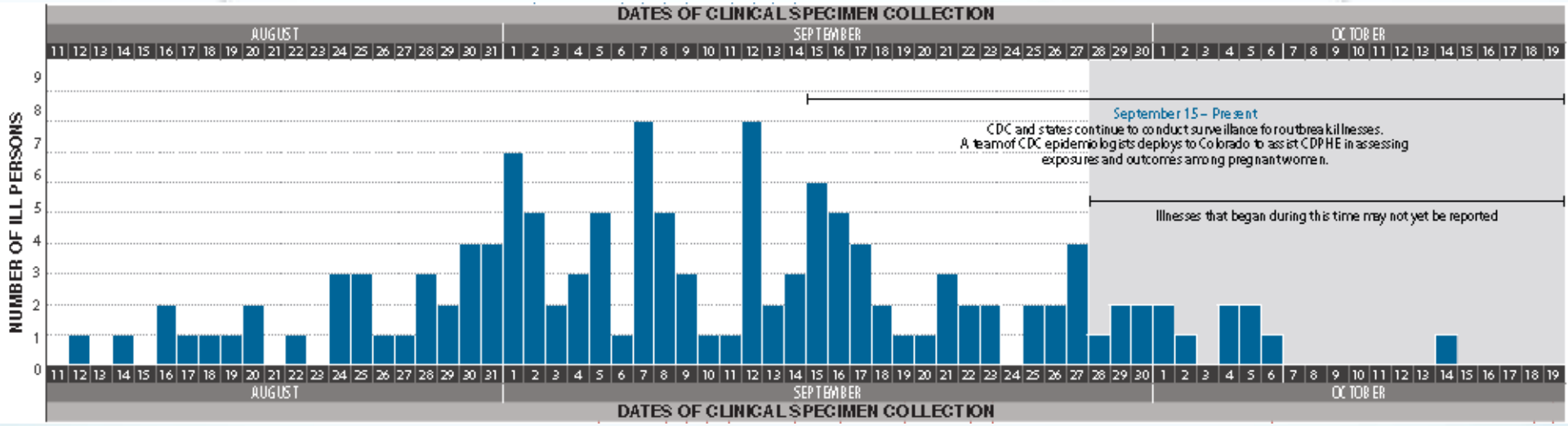

MT
1
ND
2
OR
1
ID
2
SD
1
WI
2
NY
2
WY
4
PA
1
IA
1
NV
1
NE
6
IL 3
IN
3
UT
1
CO
39
KS
10
MO
6
WV
1
VA
1
CA
2
MD
1
OK
11
AR
1
NM
15
AL
1
LA
2
TX
18
1-4 cases
5-9 cases
10-19 cases
≥20 cases

2nd Sept
Spike in Listeria Cases in Colorado

6th Sept
Pulsenet ID strains Link Cases

9th Sept
Cantaloupes ID as Source

12th Sept
Link to Jensen Farm



5th Sept
Cantaloupes Collection from homes

10th Sept
Jensen Farms Visited by FDA & COPHE

14th Sept
Jensen Farms Issue Recall

19th Sept
Environmental Samples Positive

18th Oct
FDA Issues Warning Letter to Jensen Farms

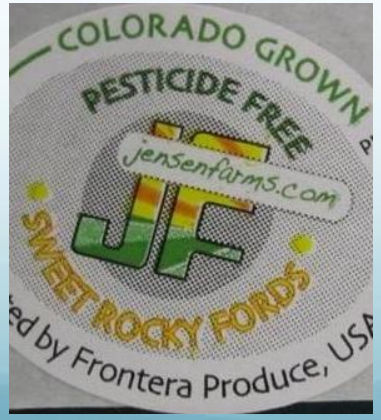
Fallout

- 3rd Party Audits: What is the value?
- Retailers: Responsibility and liability
- California Cantaloupe Producers audit checklist
- National Food Safety Guidelines

Jensen Farms

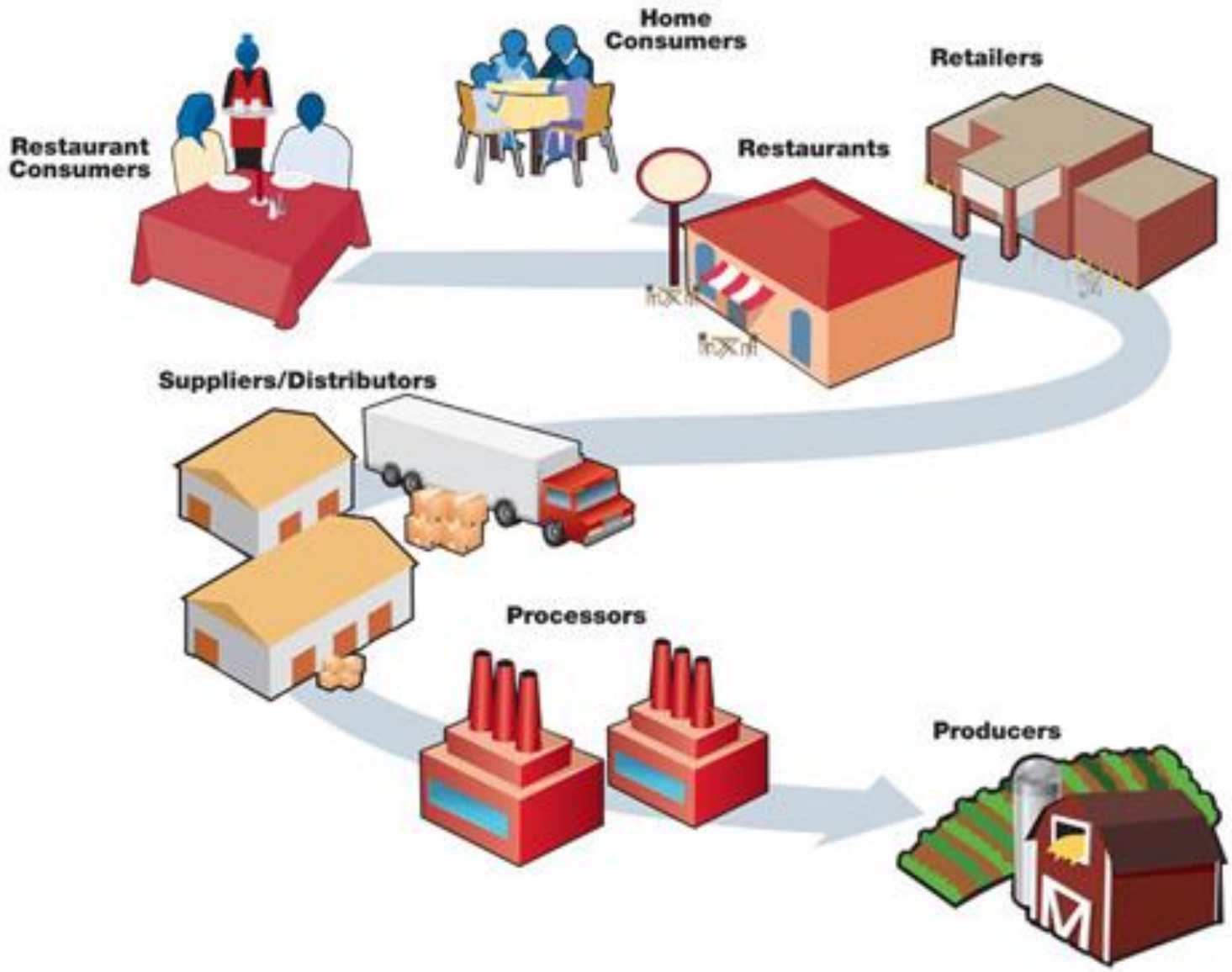
- Jensen bothers faced 6 federal charges
- 5 years Probation
- 6 Months home detention
- 100 Community service
- $150k fine
- 33 deaths 1 miscarriage

- Litigation
  - Walmart
  - Primus Labs



Year	Country	Cases	Source
2013	Australia	5 (1 death)	Profiteroles served in hospitals
2013	Australia	26 (3 deaths)	Soft Cheese
2012	New Zealand	2 (2 deaths)	Deli meats served in hospitals
2012	UK	3 (1 death)	Sandwiches Hospital

Tracing the Food Back to the Source



Sources of *Listeria*

Environment

Raw materials, Animals, milk, fish

Processing Plant

Endemic populations

Retail

Temperature abuse,
Contact surfaces

Consumer

Cross-contamination, Temperature abuse,
Storage, Use-by-date

Examples of persistence in food operations

Food	Time	Country
Cheese	4 years	Switzerland
Cheese, blue veined	7 years	Sweden
Ice cream	7 years	Finland
Smoked mussels	3 years	New Zealand
Cold smoked salmon	4 years	Denmark
Pâté	2 years	UK
Jellied pork tongue & rillettes	8 years	France
Cooked poultry	1 year	Ireland
Cooked poultry	12 years	USA

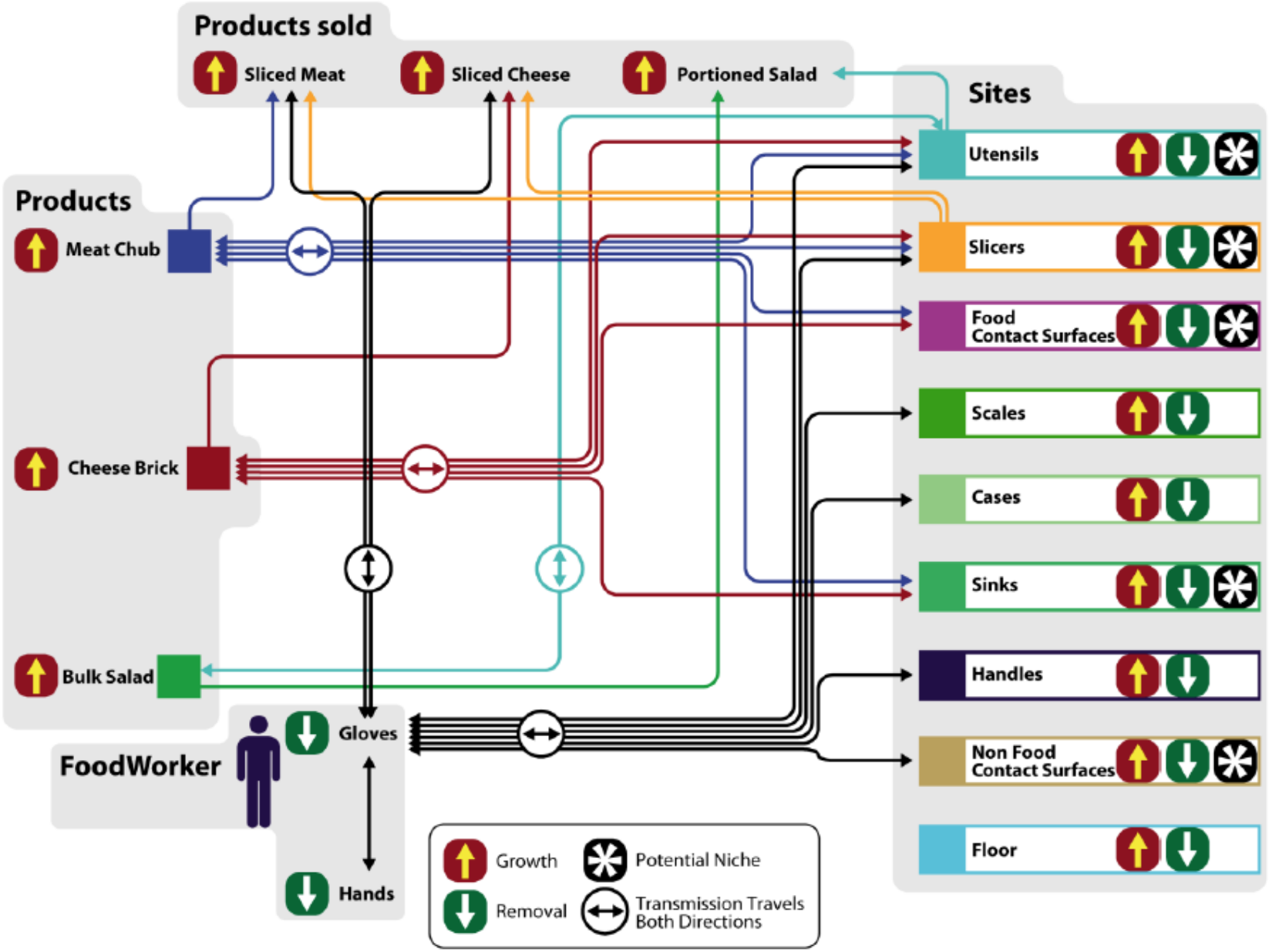


Figure 4: Diagram of "virtual deli" and cross contamination routes within the model of the Interagency Risk Assessment - *L. monocytogenes* in Retail Delicatessens.

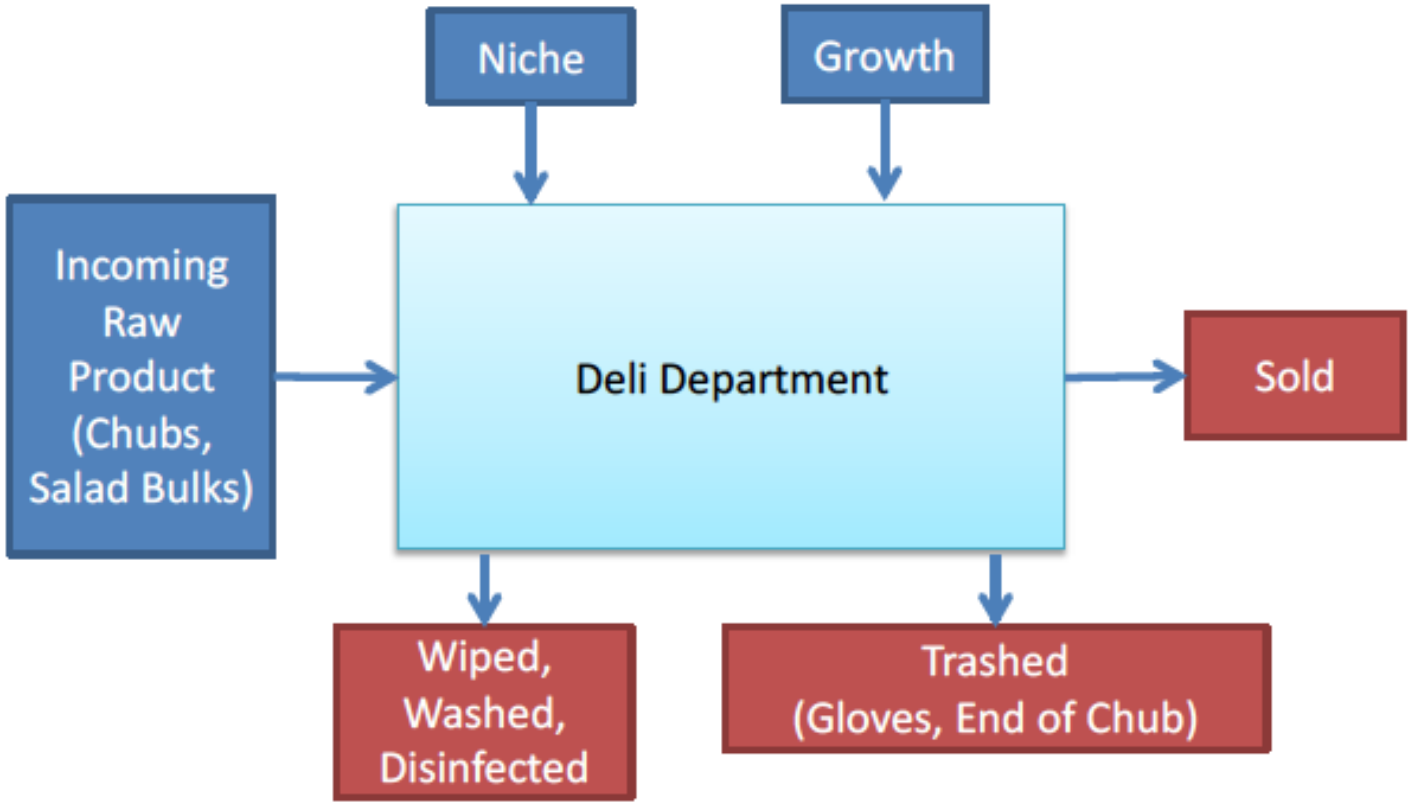


Figure 61. Incoming and outgoing bacteria in the *L. monocytogenes* in retail model.

Risk Analysis at Retail

Increased Risk

- Cross-contamination: Slicer and utensils
- Contaminated in-coming material
- Environmental niches

Temperature abuse less significant

Reduce Risk

- Anti-listeria formulation hurdles
- Frequent sanitation: surfaces and contact (slicer and utensils)
- Glove use

NSF Bugs in Your Kitchen 2013

- 20 Family homes
  - Swab sample
  - Blender
  - Can opener
  - Storage trays
  - Fridge compartments
  - Knives and cutters

Findings

Generic *E coli* (25%)

- Meat and Vegetable compartments
- Ice and water dispenser
- Storage containers

Listeria monocytogenes (10%)

Refrigerator compartment and door seal

Salmonella (25%)

- Meat and Vegetable compartments
- Blender
- Ice and water dispenser
- Pizza cutter
- Storage containers

FDA-iRisk 1.0

- Web-base delivers assessment of risk
- Model inputs
  - Hazard
  - Food product
  - Process
  - Risk scenario

Visit FDA-iRISK at
http://foodrisk.org/exclusives/fda-irisk-a-comparative- risk-assessment-tool/.

Control

Hurdle Approach to Control

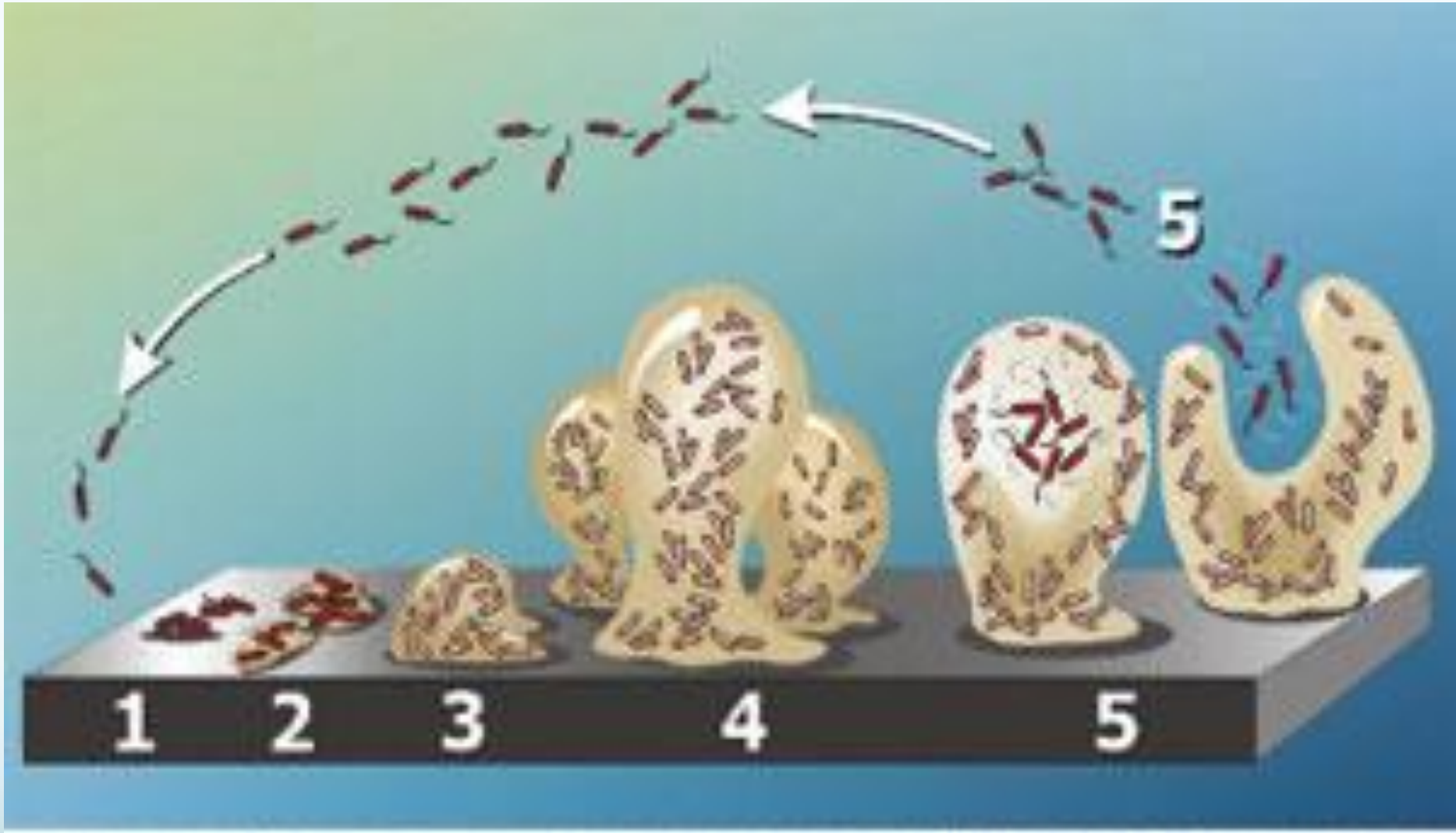
- Sanitation
- Micro-testing (products and environment)
- Physical: Pre- and Post-Pack Interventions
- Chemical: Formulation
- Biological: Bacteriophages

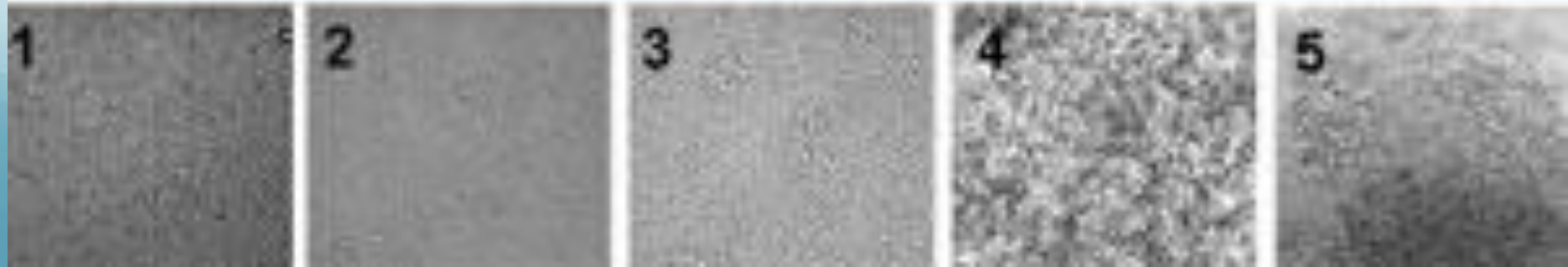
Combination effect more effective than a single intervention

Sanitation

Selection of Sanitizers

- Sanitation only effective with:-
  - Facility construction
  - GMP
  - Sanitation plan in place
  - Sanitation performed as specified in SOP


5
1
2
3
4
5


1
2
3
4
5

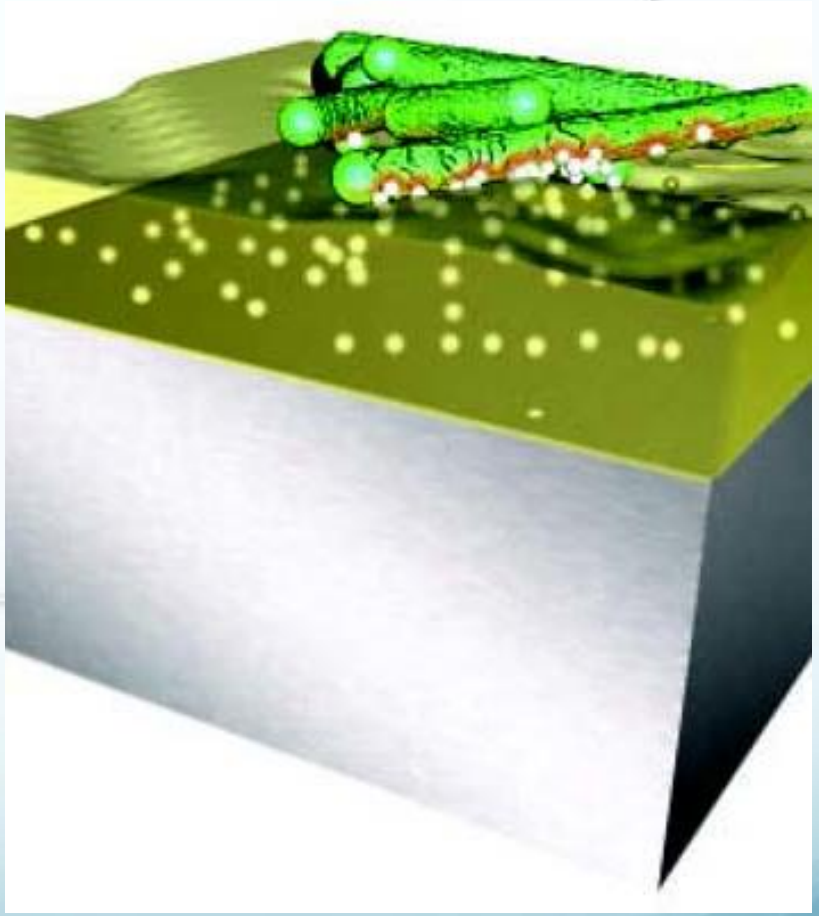
Steam Conveyor Sanitizer

Steam

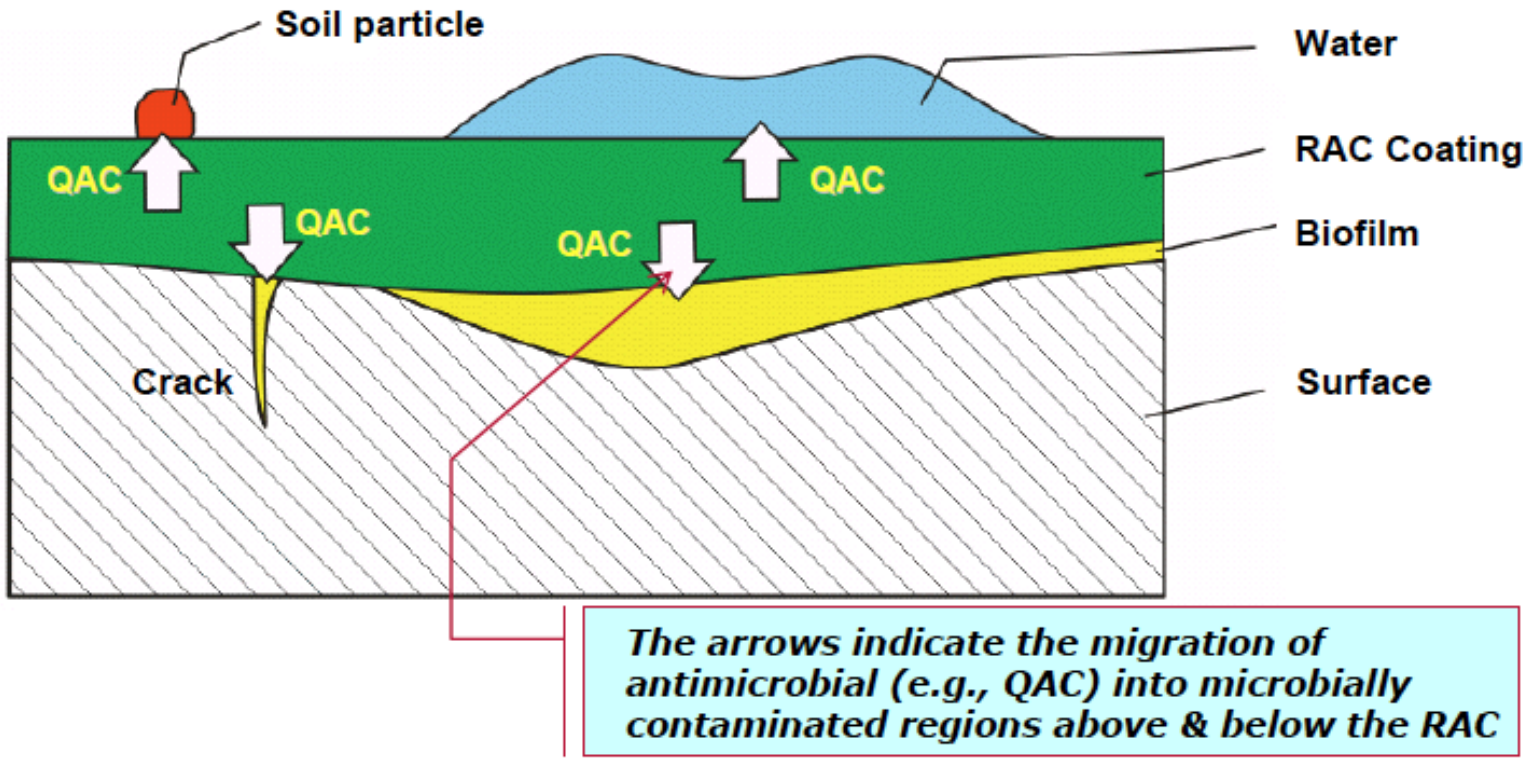
UV

Anti-microbial Coatings

- Chitosan
- Bacteriosin
- Photosensitizers (titanium dioxide)
- Titanium dioxide, Iron oxide, Barium Sulfate (Dupont Alesta)
- Silver, Copper



Renewable Antimicrobial Coatings



QAS Resistance

- Strong biofilm forming ability
- Resistance based on efflux pump
- Linked to antibiotic resistance
- Sub-lethal concentration of QAS
- *Listeria* strains in Maple Leaf deli meats harbored genes encoding for efflux pumps.

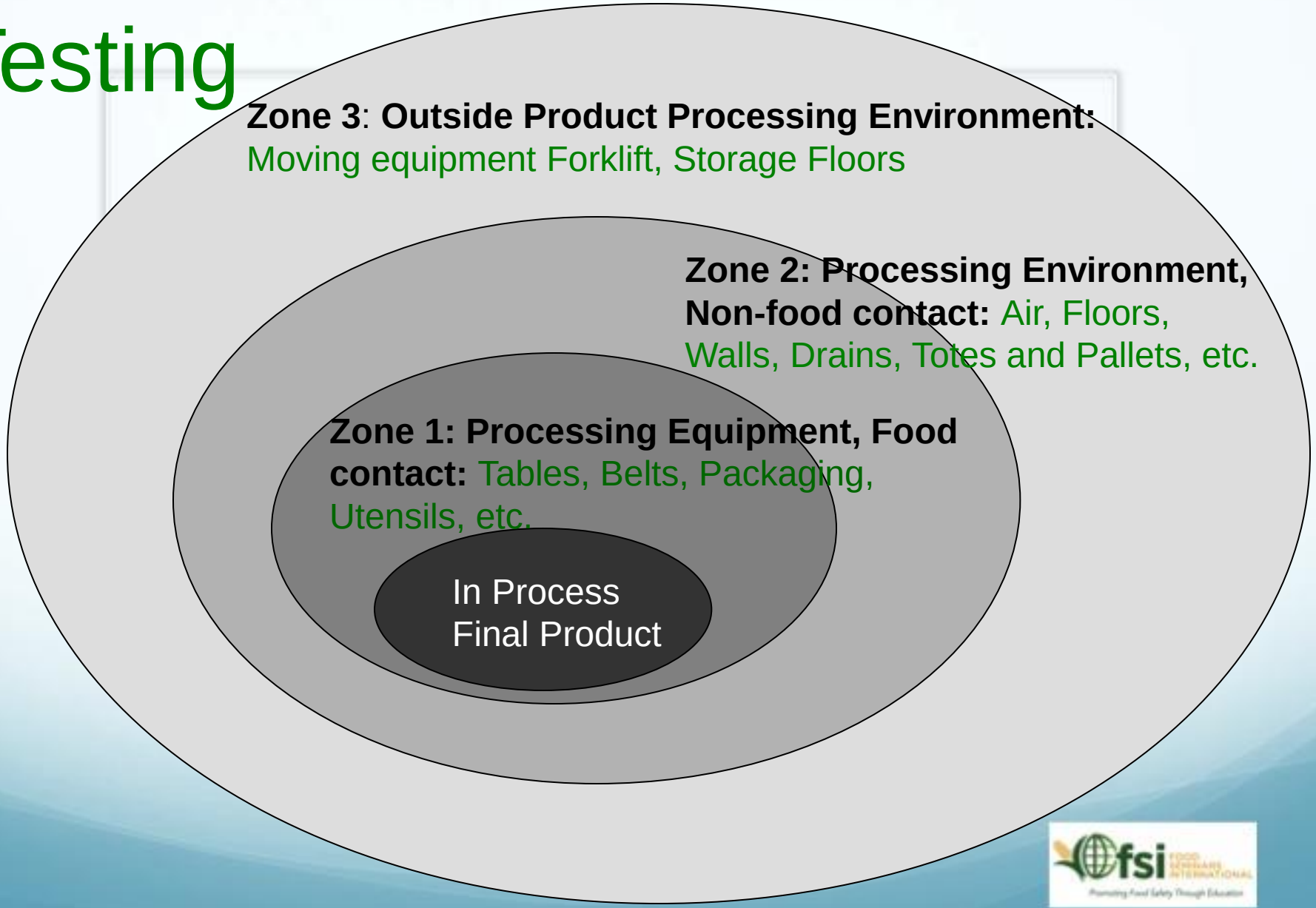
Sanitizer Rotation

- Example, switch from QAS to hydpochlorite then to iodine
- Start rotation when *Listeria* repeatedly found in environmental sampling
- Possible to rotate sanitizers every month

Sanitizers Effective Against *Listeria*

- Chlorine containing compounds (hypochlorite)
- Quaternary ammonium salts
- Acid sanitizers (organic acids, hydrogen peroxide:acetic acid)
- Iodophores
- Ozone

Zone Testing



When to Test

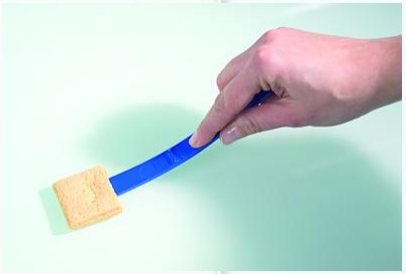
- Pre-processing
  - Identify problems with sanitation

Mid-operation

-Identify problems with the product.

Environmental Monitoring Procedure – Surface Sampling

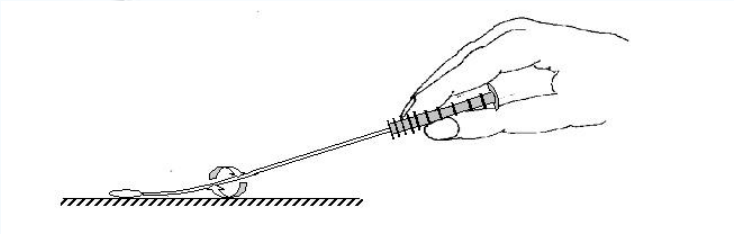
- Sponges
  - Sponges are generally used for monitoring pathogens in wet environments. By using a sponge, you can sample large surface areas



- Swabs
  - Stick swa  used for monitoring bacteria. They can also be used for searching for pathogens in hard-to-reach sites like crevices or threads.

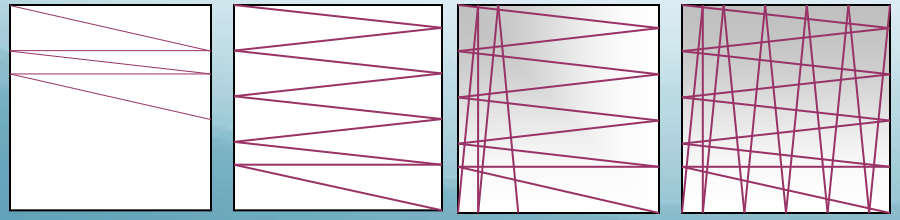
Swab

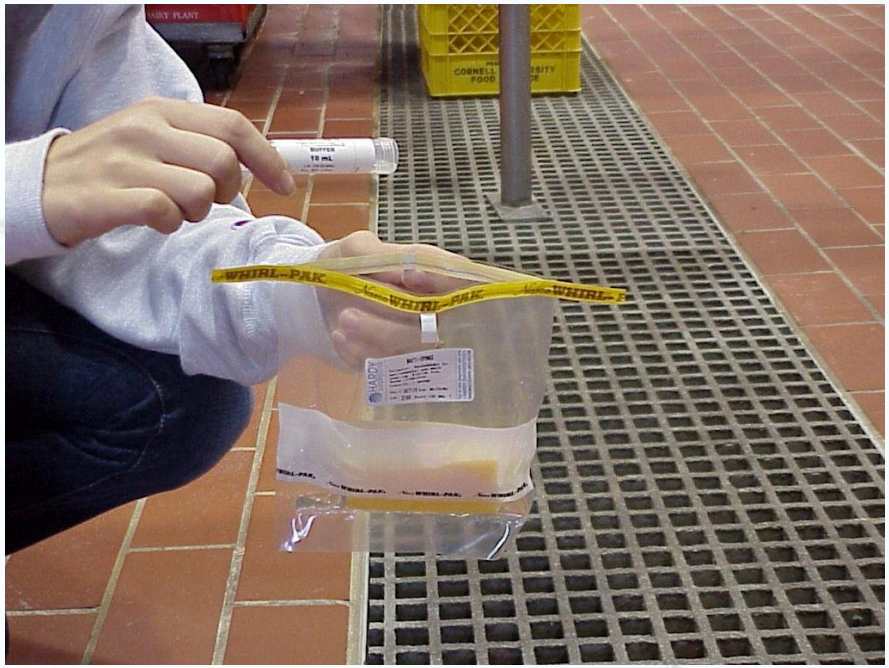
Small crevices or hard to reach areas



Sponge

- *Large areas*
- *Uneven surfaces*
- *High spots, deep containers, etc.*

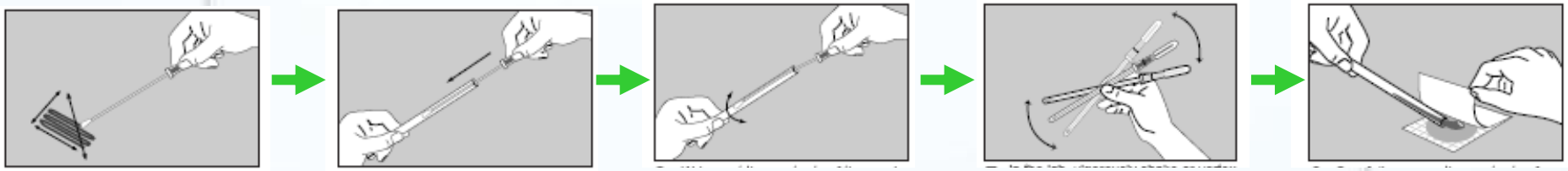


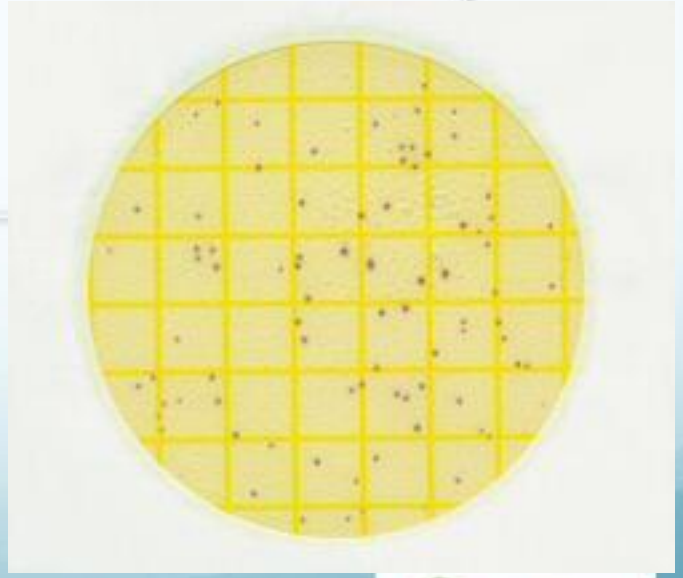

WHIRL-PAK


THIS
SIDE
UP
WHIRL-PAK

Rapid Sanitation Monitoring

SWAB





Listeria spp as an indicator for *Listeria monocytogenes*

Regulations

Timeline

1929 — First Isolation *Listerella*

1940 — Renamed *Listeria*

1979 — Recognized as an Emerging Pathogen

1982 — Confirmed as a Foodborne Pathogen

1987 — Zero Tolerance Policy

Codex Guidlines

- Attempt to form a global standard

Provide a framework for government to review polices

Reduce the risk of listeriosis in the population

Ensure fair practices in food trade

Codex Listeria Standard 2009

- RTE Foods that cannot support the growth of LM: 100 cfu/g

- Zero tolerance for RTE foods that can support growth

  Change in LM levels by 0.5 log cfu/g over shelf-life of product

Regulations

No Global Standard for RTE Foods

- Zero Tolerance
  - US
  - New Zealand
  - Australia
  - Austria
  - Italy

  Not Detected in 25g

Alternative 1

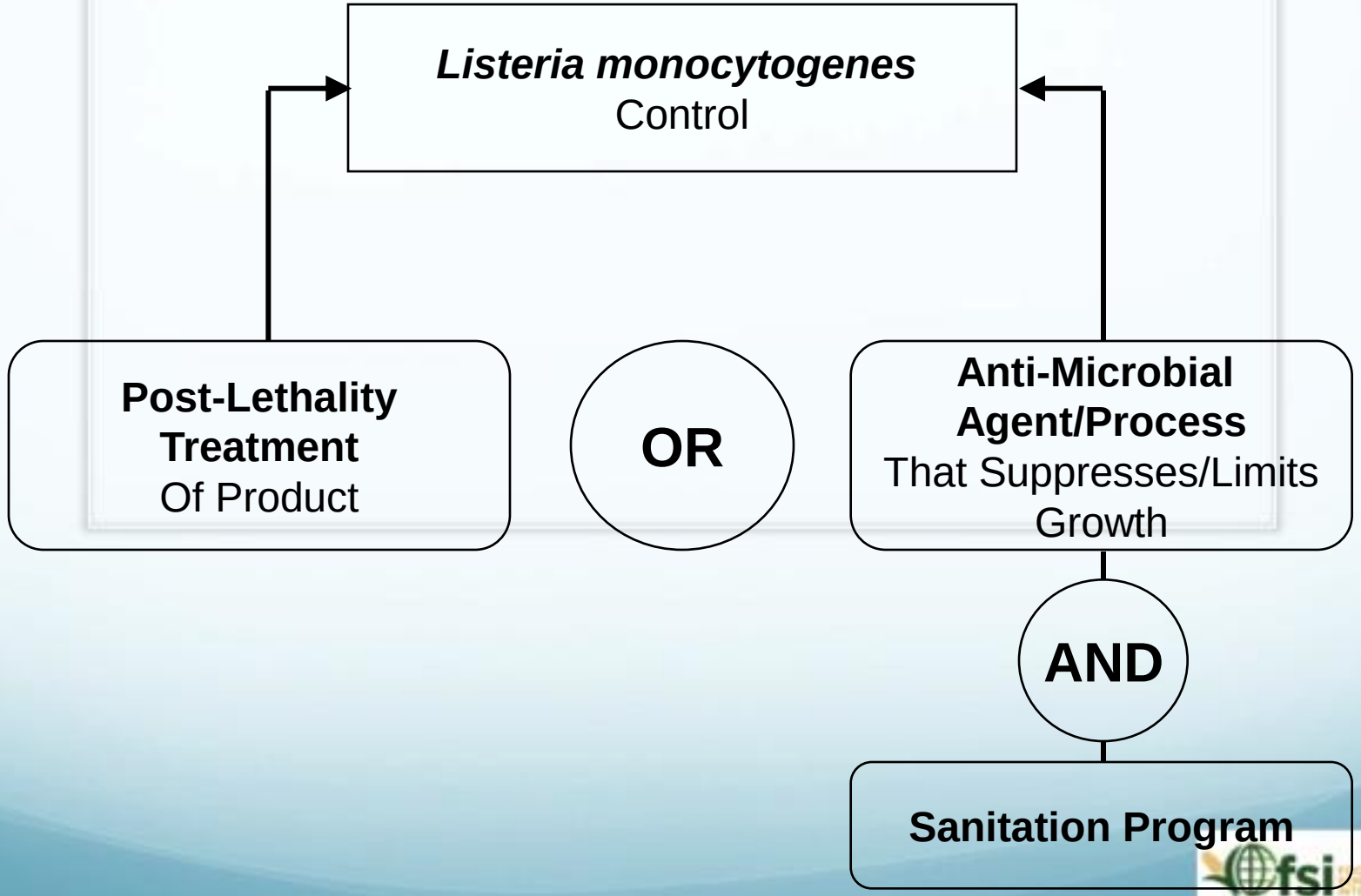
Listeria monocytogenes
Control

Post-Lethality Treatment
Of Product

AND

Anti-Microbial Agent/Process
That Suppresses/Limits Growth

Alternative 2



Alternative 3

Use of sanitation measures only

* May have post-lethality treatment and/or antimicrobial agent/process but not documented as being sufficient to provide enhanced safety

Hold-and-Release for deli and hotdog products

FDA vs USDA

	USDA	FDA
Alternative 1 Post-exposure lethality treatment AND anti-microbial agent.	2 food contact sites / line / 6 months (Voluntary.)	5 food contact sites / line / week. Test all identified FCS / month. No differentiation between product type/category. (Voluntary.)
Alternative 2 Post-exposure lethality treatment OR anti-microbial agent.	2 food contact sites / line / quarter (Mandatory.)	
Alternative 3 (Sanitation only to control *L. monocytogenes*.)	1-4 food contact sites / line / month, depending on product and size of operation. (Mandatory.)	

USDA *Listeria* Control Guide

- April 2014
- Focused on retail

- Product handling
- Sanitation
- Facility and equipment
- Working practice
- Self Assessment tool

Risk Based

- Tolerance limits
  - Germany
  - France
  - Holland

<100 cfu/g up to the point of sale

Canada

EU Criteria for *L. monocytogenes* in Foods

Food Category	Sampling Plan	Limits	Where Criterion Applies
RTE Foods Intended for Infants and SMP	N = 10 C = 0	Neg/25g	Products in the market
RTE Able to Support Growth of LM (Excluding intended for infants and SMP)	N = 5 C = 0 N = 5 C = 0	100 cfu/g Neg/25g	Products in the market Before leaving products in the processor
RTE foods unable to support growth of LM (Exclude intended for infants and SMP	N = 5 C = 0	100 cfu/g	Products in the market

SMP: Special Medical Purposes

Listeria monocytogenes
Health Canada

Category	Definition	Example Foods
Category 1	Support the growth of *Listeria*	Deli-meats Soft-Cheese Hot dogs Pate
Category 2A	Limited growth of Listeria <0.5 log cfu/g within shelf-life No kill step, and/or Shelf-life <5 days	Smoked ham Fresh Produce
Category 2B	Do not support the growth of Listeria	Ice cream Hard Cheese Dry salami

Health Canada *Listeria* Challenge Studies

- Potential for growth of *Listeria monocytogenes* growth
- How to assess the lethality of treatments applied to inactivate *Listeria monocytogenes*.

Challenge Testing

- 3-5 strains (up to 10): 1/2a, 1/2b and 4b
- Surrogate: *L. innocua*
- Pre-adapt to stress: Temperature, pH
- Lethality treatment:
  - 6-7 log cfu/g
  - 3 log cfu/g: confirm complete inactivation
- Growth Challenge
  - 10-30 cfu/g : <100 cfu/g at the end of shelf-life

Growth Challenge Studies

- Temperature abuse: 7° C
- Aim for 1.5 times the shelf-life
- 5 batches of product

- Report
- Justify strain selection and preparation
- Methods and storage conditions
- Statistical analysis

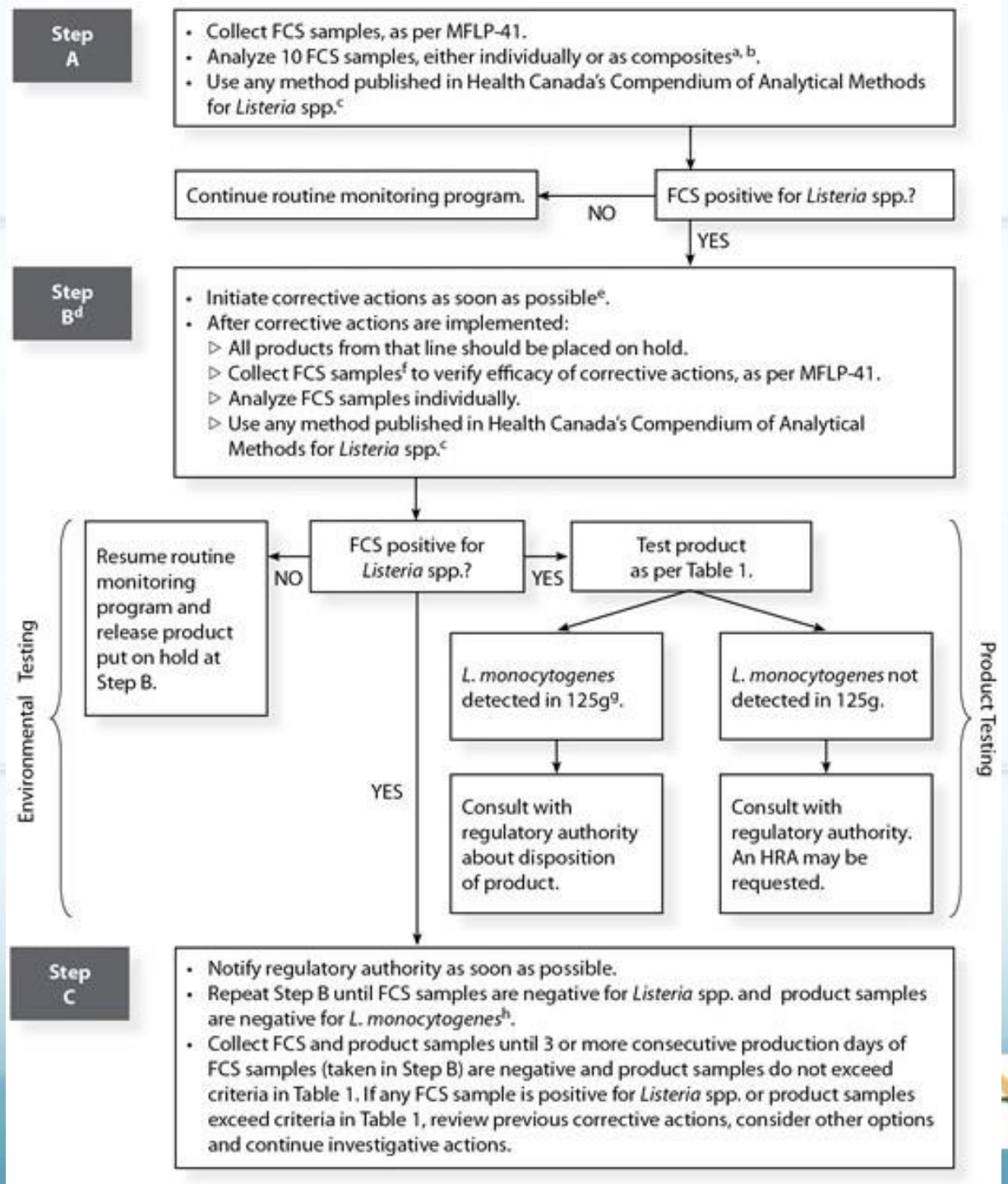
Category	Testing Requirements	Criteria	Level of Inspection
Category 1	5 sample units 100g 5x25g Final product	Negative by enrichment	High
Category 2A	5 sample units 100g 5 x 10g Final Product	<100 cfu/g	Medium-low
Category 2B	5 sample units 100g 5 x 10g Final Product	<100 cfu/g	Low
Exempt	>5 log cfu reduction in *Listeria* (thermal not HHP) Fresh fruit and vegetables Raw meat and fish (except sushi) Not RTE		

Category 1

- Increased testing
- Zero tolerance
- Positive highly likely to trigger recall
- Interventions to reduce or prevent growth of Listeria
- Priority for inspection

- Focus of transferring from Cat 1 to Cat 2A

Category 1


Step A
Collect FCS samples, as per MFLP-41.
Analyze 10 FCS samples, either individually or as composites[a, b].
Use any method published in Health Canada's Compendium of Analytical Methods for Listeria spp.[c]
FCS positive for Listeria spp.?
NO
Continue routine monitoring program.
YES
Step B[d]
Initiate corrective actions as soon as possible[e].
After corrective actions are implemented:
All products from that line should be placed on hold.
Collect FCS samples[f] to verify efficacy of corrective actions, as per MFLP-41.
Analyze FCS samples individually.
Use any method published in Health Canada's Compendium of Analytical Methods for Listeria spp.[c]
FCS positive for Listeria spp.?
NO
Resume routine monitoring program and release product put on hold at Step B.
YES
Test product as per Table 1.
L. monocytogenes detected in 125g[g].
L. monocytogenes not detected in 125g.
Consult with regulatory authority about disposition of product.
Consult with regulatory authority. An HRA may be requested.
Environmental Testing
Product Testing
YES
Step C
Notify regulatory authority as soon as possible.
Repeat Step B until FCS samples are negative for Listeria spp. and product samples are negative for L. monocytogenes[h].
Collect FCS and product samples until 3 or more consecutive production days of FCS samples (taken in Step B) are negative and product samples do not exceed criteria in Table 1. If any FCS sample is positive for Listeria spp. or product samples exceed criteria in Table 1, review previous corrective actions, consider other options and continue investigative actions.

Decision Tree Cat 1

10 Food Contact Surfaces (FCS)

Composite or single screening for LM

Negative → Continue Routine Screening

Positive ↓

Implement Corrective Action
- Hold Product
- Collect 10 FCS screen individually
- Start investigation

Negative → Release Product
Continue Routine Sampling

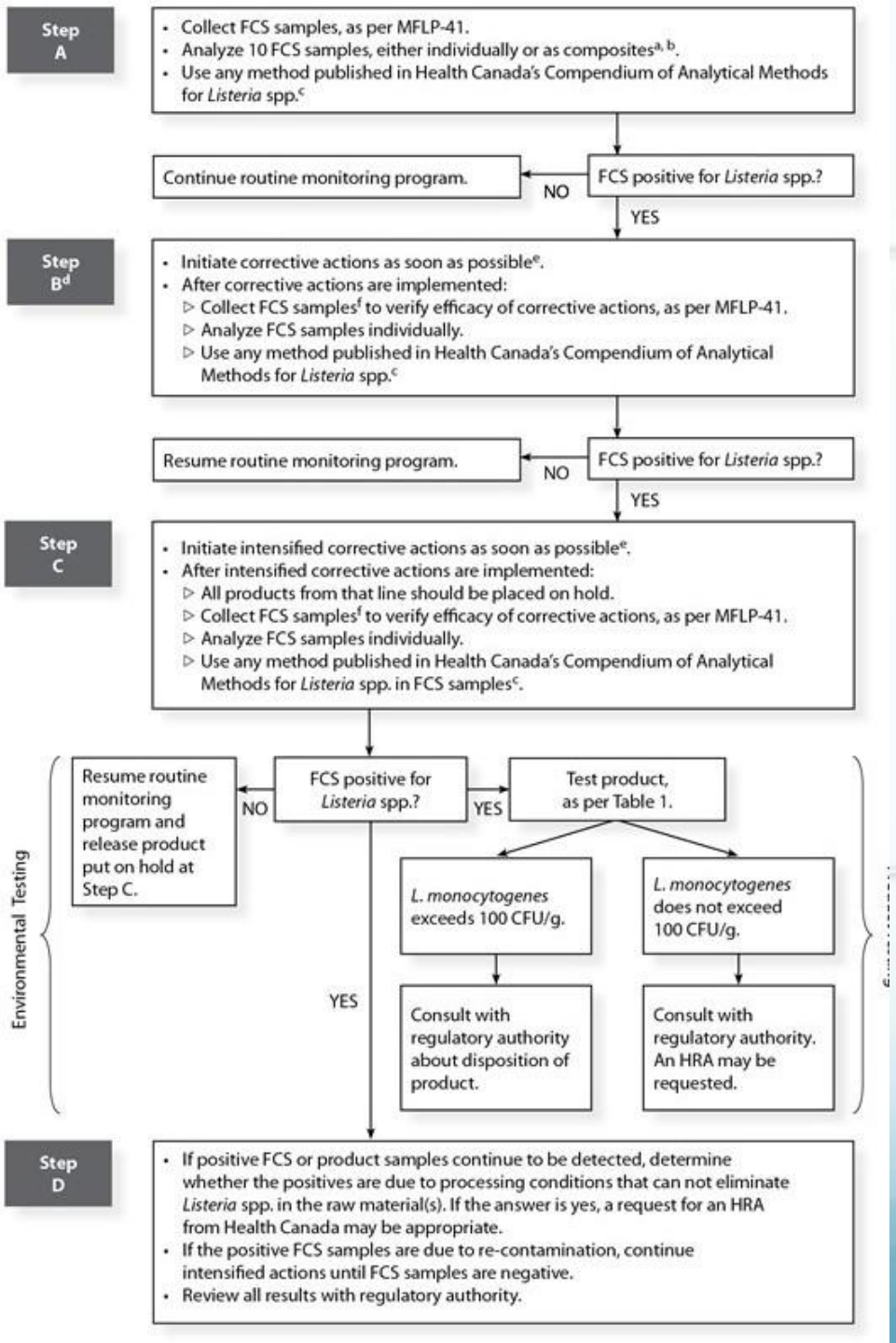
Positive → Contact regulator
Review sanitation
Repeat FCS sampling until 3 negatives
Review results with regulator

Positive → Screen 125g Product

Positive → Contact Regulators
Dispose Product

Negative → Consult Regulators
Request risk assessment

Category 2


Step A
Collect FCS samples, as per MFLP-41.
Analyze 10 FCS samples, either individually or as composites[a, b].
Use any method published in Health Canada's Compendium of Analytical Methods for Listeria spp.[c]
FCS positive for Listeria spp.?
NO
Continue routine monitoring program.
YES
Step B[d]
Initiate corrective actions as soon as possible[e].
After corrective actions are implemented:
Collect FCS samples[f] to verify efficacy of corrective actions, as per MFLP-41.
Analyze FCS samples individually.
Use any method published in Health Canada's Compendium of Analytical Methods for Listeria spp.[c]
FCS positive for Listeria spp.?
NO
Resume routine monitoring program.
YES
Step C
Initiate intensified corrective actions as soon as possible[e].
After intensified corrective actions are implemented:
All products from that line should be placed on hold.
Collect FCS samples[f] to verify efficacy of corrective actions, as per MFLP-41.
Analyze FCS samples individually.
Use any method published in Health Canada's Compendium of Analytical Methods for Listeria spp. in FCS samples[c].
FCS positive for Listeria spp.?
NO
Resume routine monitoring program and release product put on hold at Step C.
YES
Test product, as per Table 1.
L. monocytogenes exceeds 100 CFU/g.
L. monocytogenes does not exceed 100 CFU/g.
Consult with regulatory authority about disposition of product.
Consult with regulatory authority. An HRA may be requested.
Environmental Testing
YES
Step D
If positive FCS or product samples continue to be detected, determine whether the positives are due to processing conditions that can not eliminate Listeria spp. in the raw material(s). If the answer is yes, a request for an HRA from Health Canada may be appropriate.
If the positive FCS samples are due to re-contamination, continue intensified actions until FCS samples are negative.
Review all results with regulatory authority.

Decision Tree Cat 2

10 non-Food Contact Surface (non-FCS)

Screen Composite for *Listeria* spp

Negative → Continue Routine Sampling

Positive

Corrective Action (e.g. sanitation)
Collect 10 non-FCS samples and screen individually for *Listeria* spp

Positive

Repeat Corrective Action unit Negative tests on non-FCS
IF non-FCS is Close to FCS then take Samples for FCS and Screen for *Listeria* spp

Listeria Environmental Results

- Trend analysis
- Revise sanitation plans (sanitizer rotation)
- Track source of *Listeria*

Control

- Chemical

- Biocontrol

- Physical

Product Formulation

Growth Parameters of *L. monocytogenes*

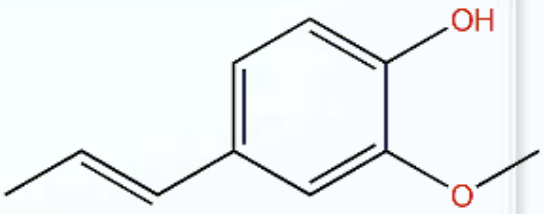
Parameter	Critical limit for LM growth
pH	4.4
NaCl	13%
Aw	0.92
Temperature	1-45° C
Nitrite (curing)	>800ppm (bacteriostatic)
Oxygen	Facultative anaerobe

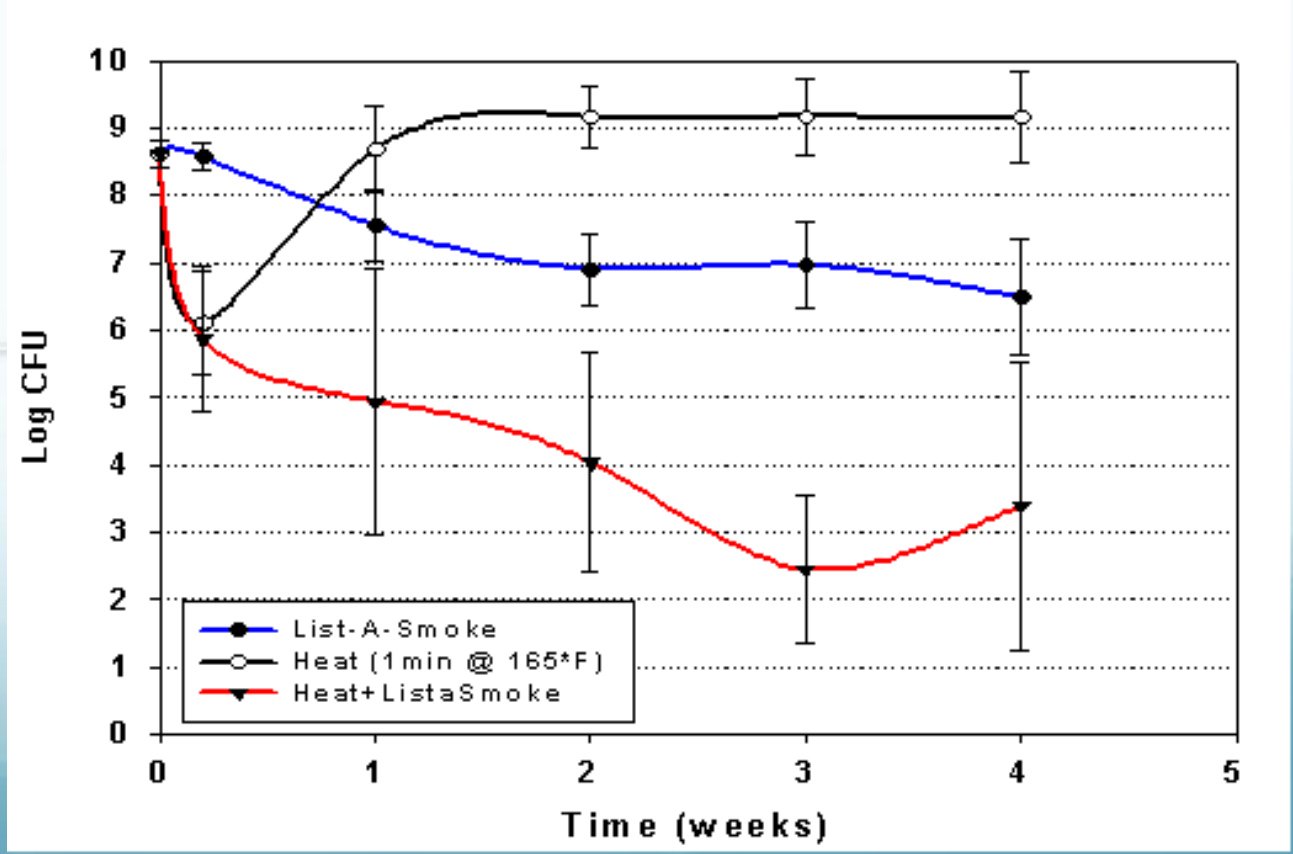
Smoke/Liquid Smoke

- Applied as a spray or dip

  - Eugenol/Isoeugenol

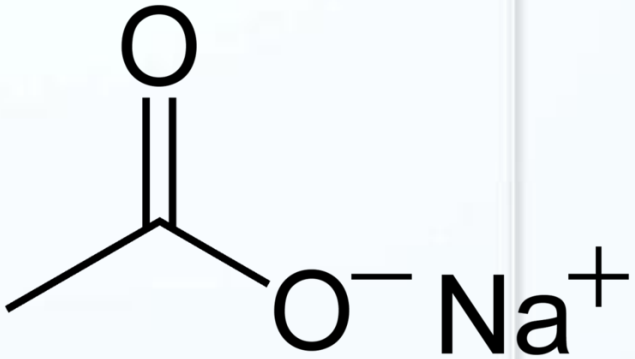
  -Phenolics





Sodium Diacetate

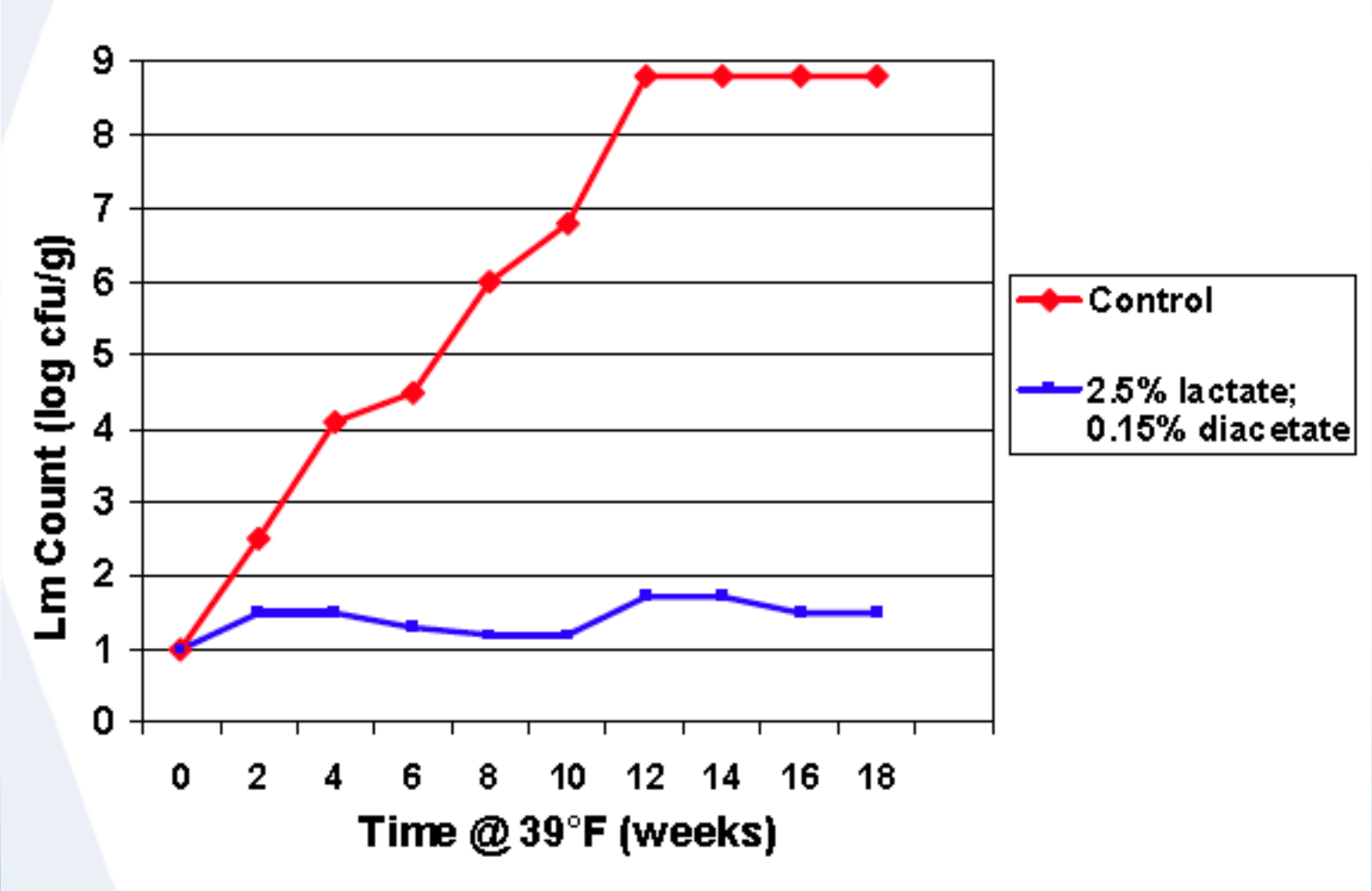
60% Sodium acetate
40% Acetic acid



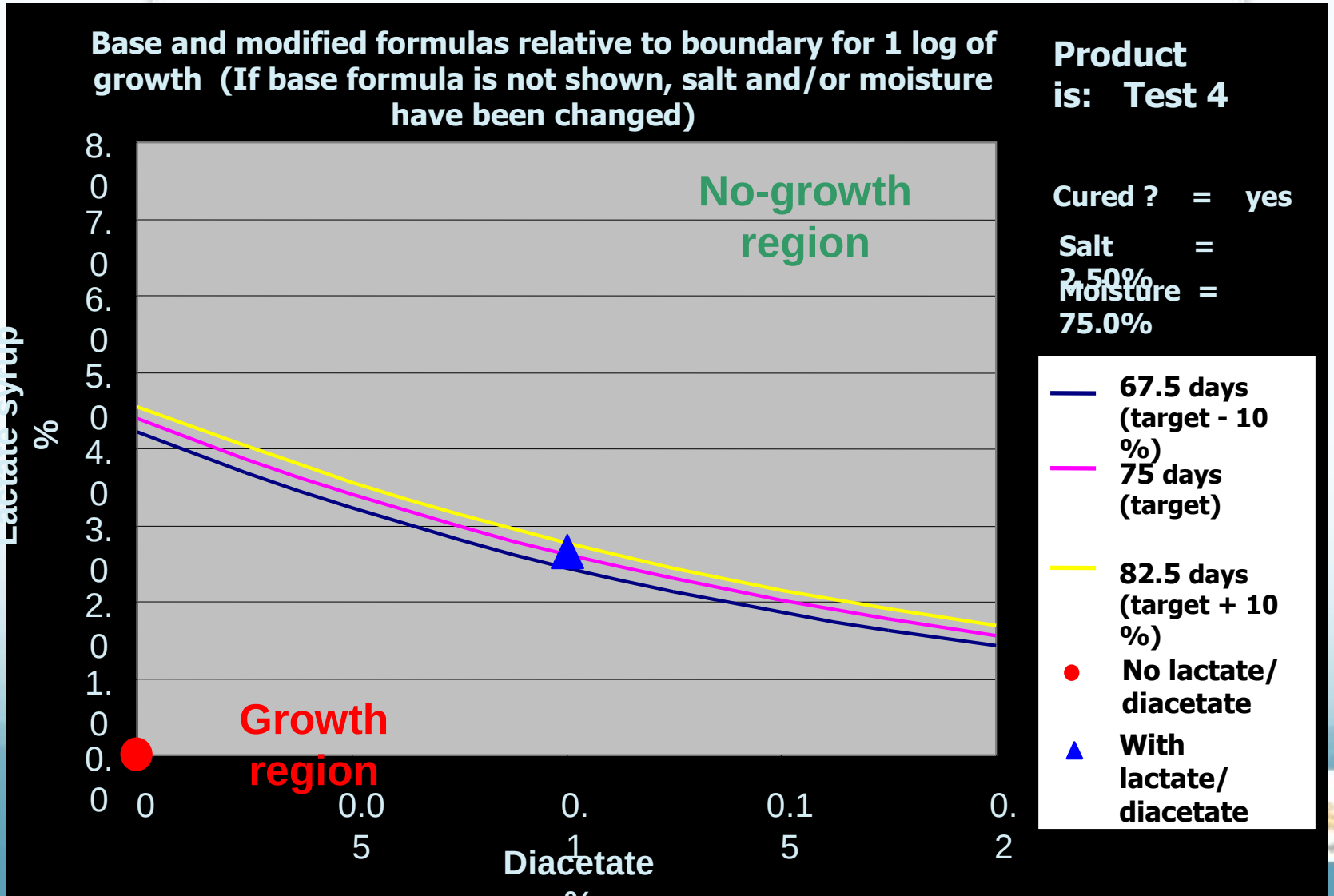
Sour flavor

Used in Combination with Pottassium or sodium lactate

Upper limit 0.2% but more commonly 0.1- 0.15%


Lm Count (log cfu/g)
9
8
7
6
5
4
3
2
1
0
0
2
4
6
8
10
12
14
16
18
Time @ 39°F (weeks)
Control
2.5% lactate;
0.15% diacetate

Lactate/Diacetate



Plant Extracts

- Eugenol
- Rosemary
- Horseradish distillate

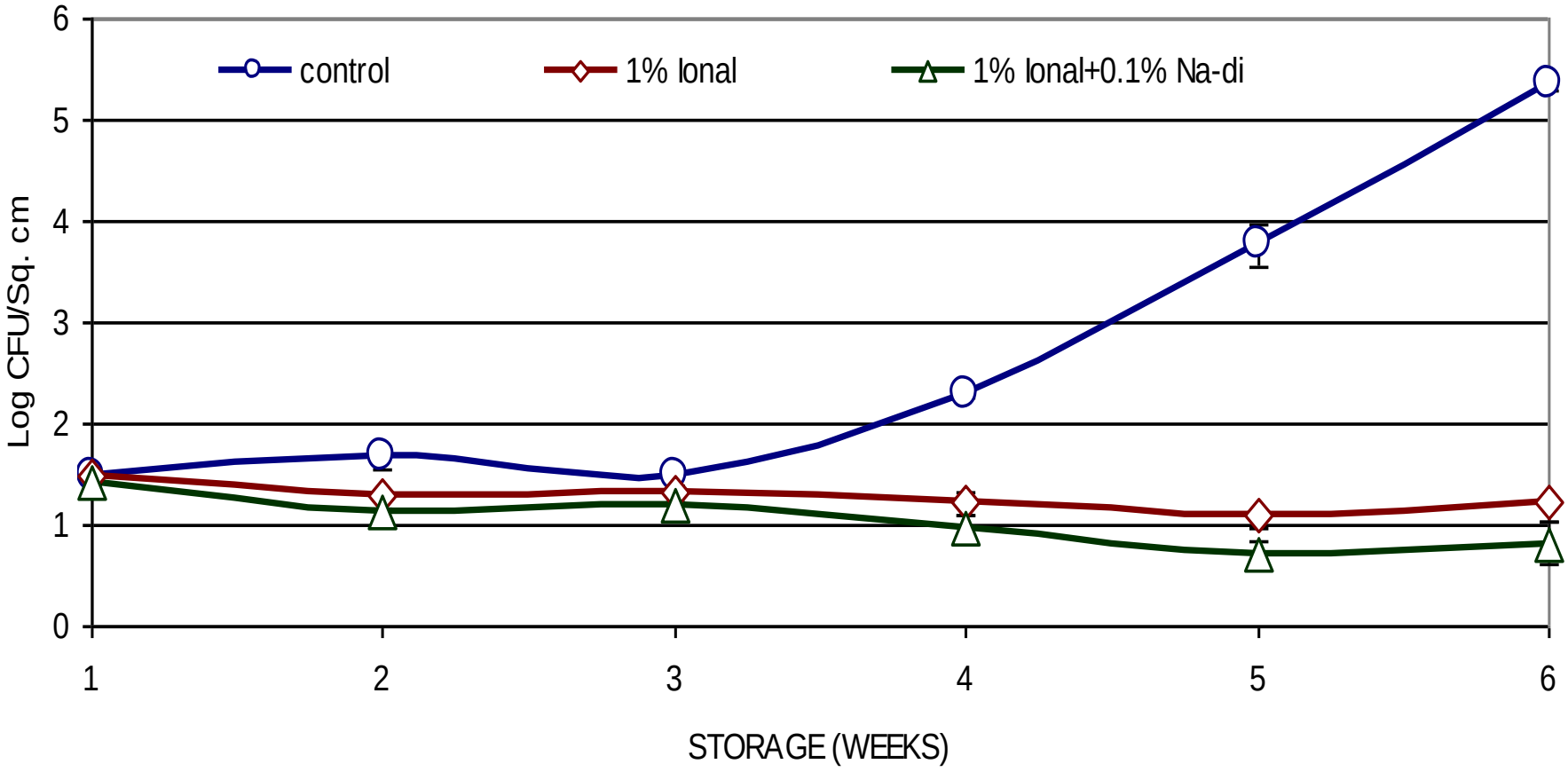
- Impact on sensory characteristics
- Less effective in products compared to in vitro

Other Preservatives

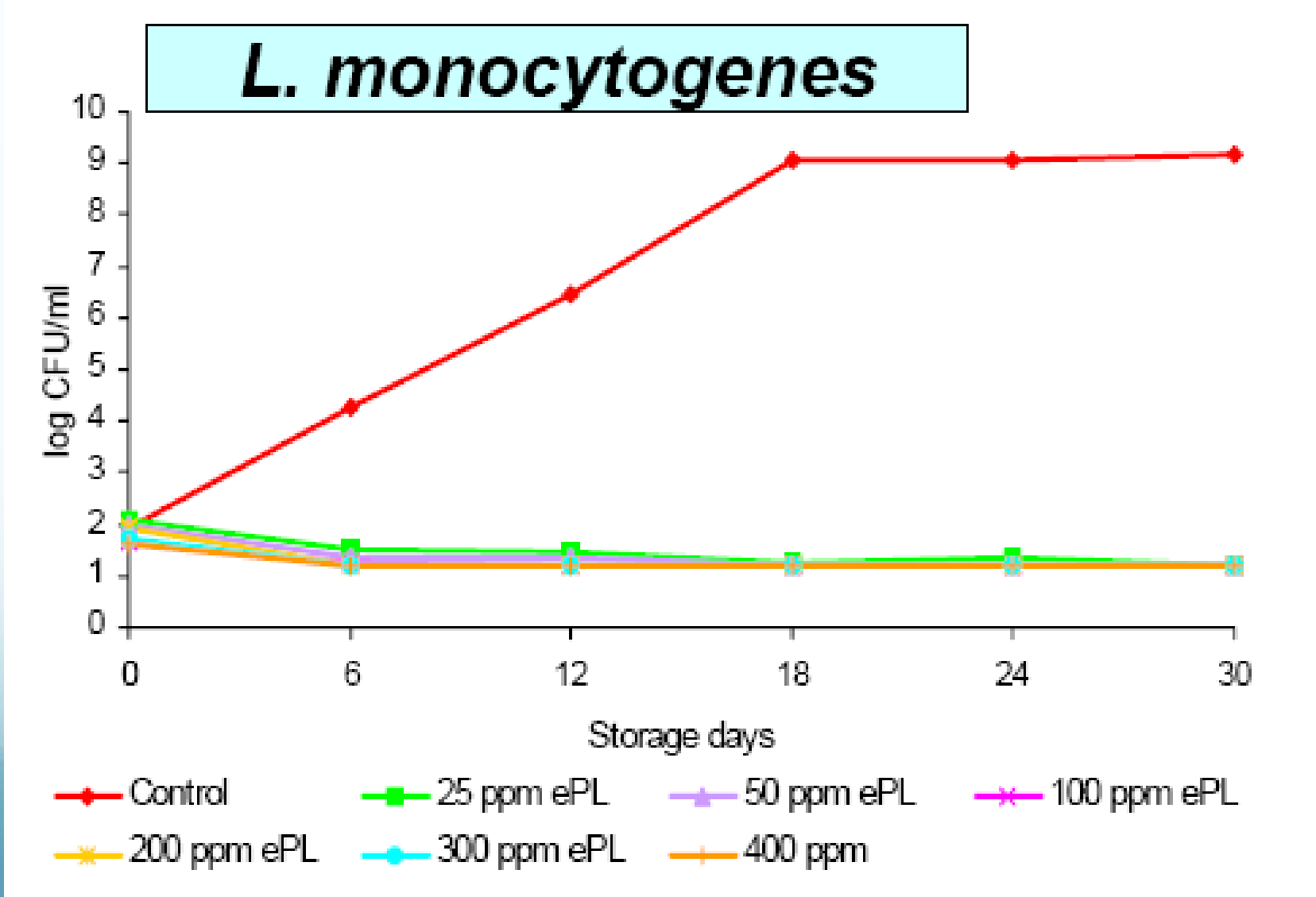
- Sorbic acid: Use in combination with low < pH 5 and temperature <(5C).
- Bacterocins: Nicin, Paraben
- Poly L-lysine
- Citrate:EDTA when used in combination with other preservatives
- Monogycerides (dip or spray): Bacterocidal when combined with organic acids
- Cultured dextrose

Citrate Dip

Inhibition of Growth of *L. monocytogenes* at 4° C by Buffered Sodium Citrate (Ional™)



Poly L-lysine



Cultured Dextrose or Cultured Skimmed Milk

- Cell-free spent culture media of probiotic bacteria

  Organic acids

  Bacteriocins

  Peptides (anti-adhesion factors)

Danisco e.g. MicroGUARD

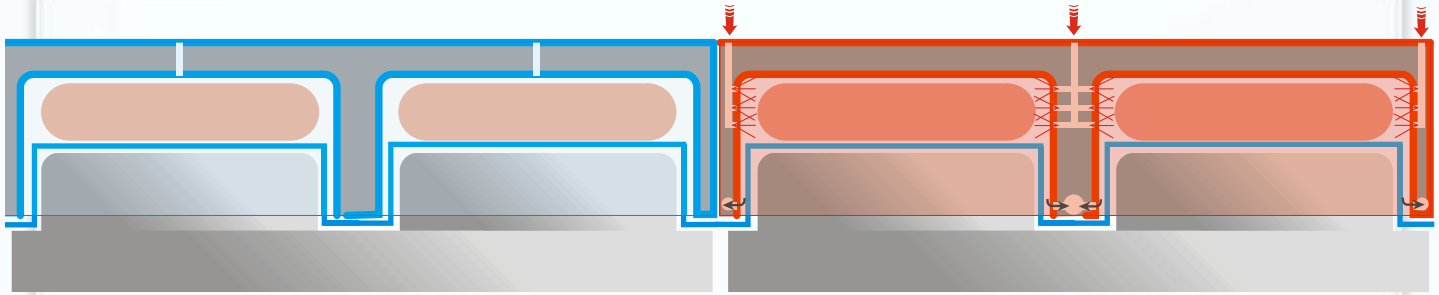
Physical Interventions

- In-pack pasteurization
- Immediately before packaging

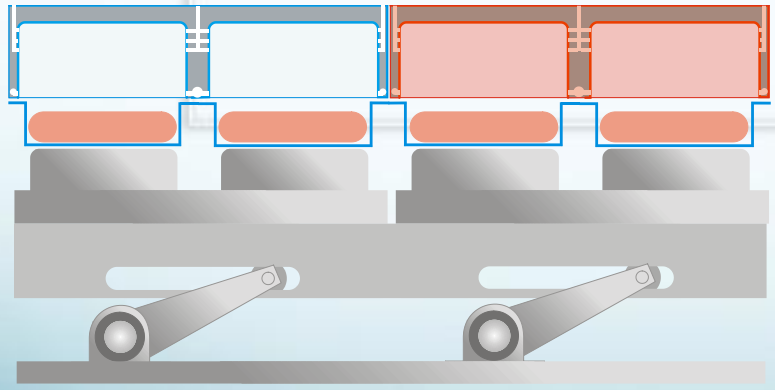
- Used in combination with product formulations that prevent the growth of *L. monocytogenes*

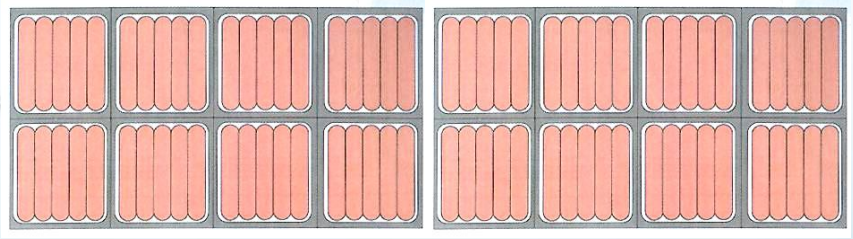
Pre-Pack Steam Surface Pasteurization

Steam Surface Pasteurization



Vacuum Cooling **Steam Injection**





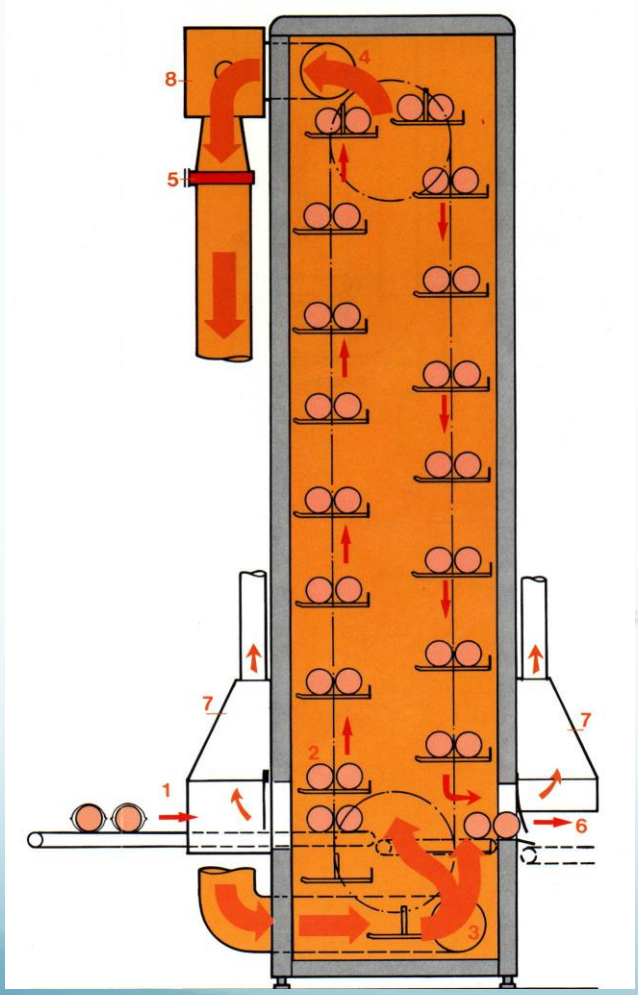
Servo lifts product pedestal up into SSP chamber

ALKAR-RapidPak, Inc.

Steam Surface Pasteurization

- Hot dogs inoculated with indicator microorganism
- SSP treatment = 1.5 seconds
- 4-log reduction for single-layer package configuration
- Actual *Listeria monocytogenes* inoculated-pack tests (10^2 per package) resulted in zero positives

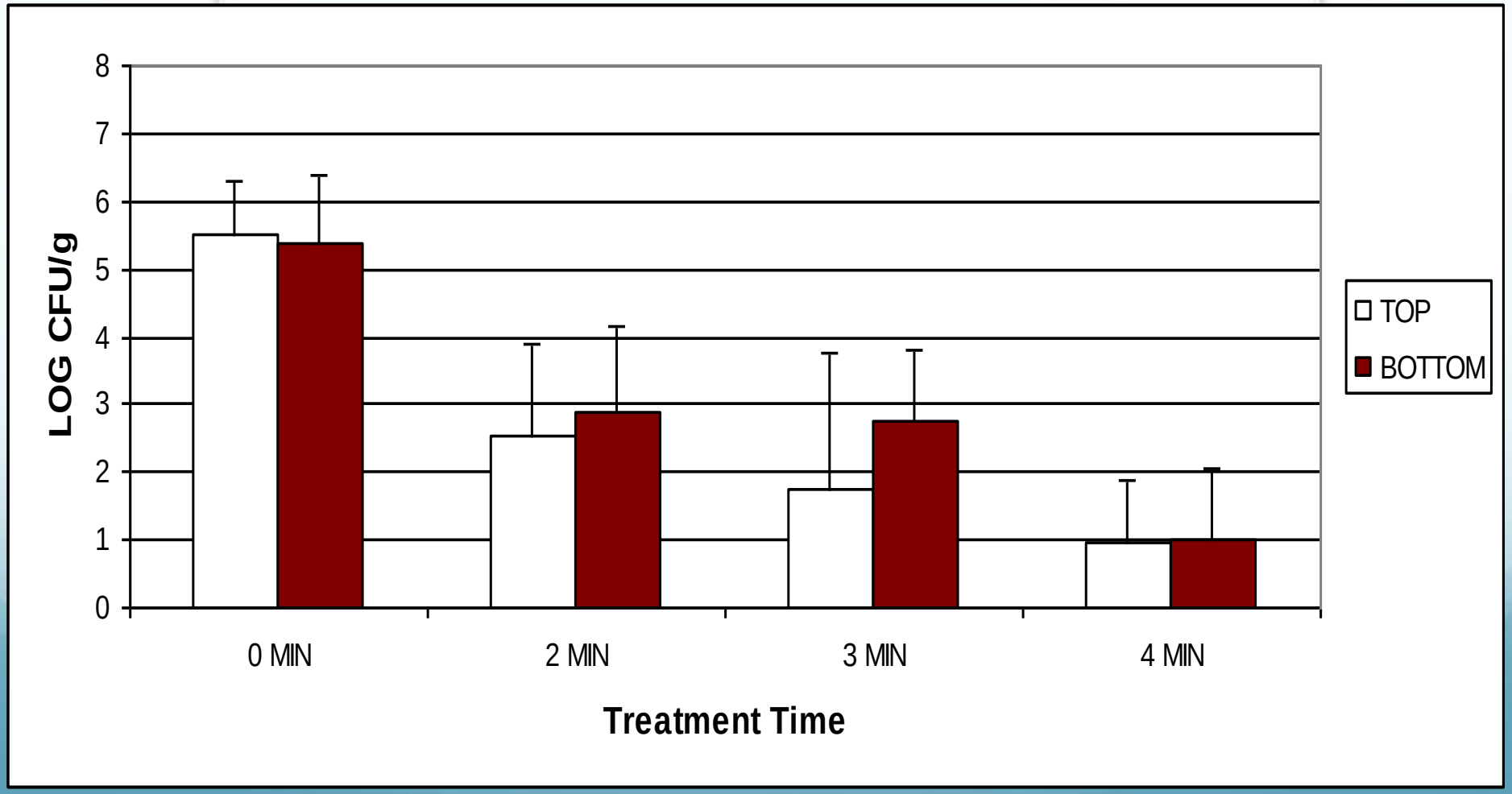
In-Package Steam Pasteurization



Stork RMS-Protecon (Townsend) Steam-Based Post-Process Pasteurization System

In Package Pasteurization

Log cycle reduction of *Listeria monocytogenes* at 96° C



High Pressure Processing

Gridpath Solutions Inc

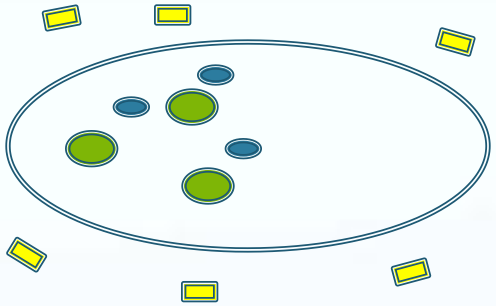
NC Hyperbaric

Diverse Range of Products can be HHP

Mode-of-Inactivation

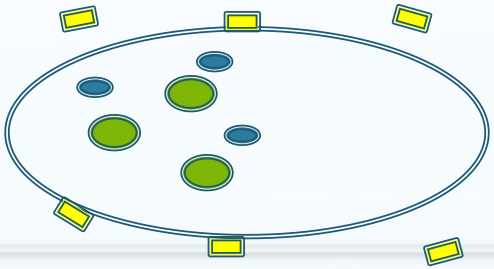
400 MPa

Irreversible



Enzyme denaturation, ATP generation inhibited, membrane destabilization

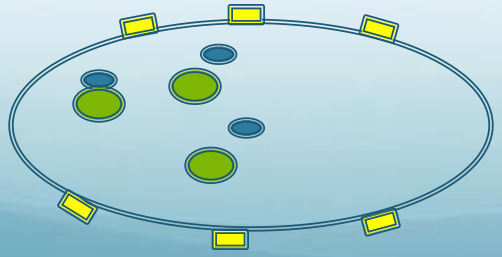
300 MPa



Porin's dissociate from cell membrane

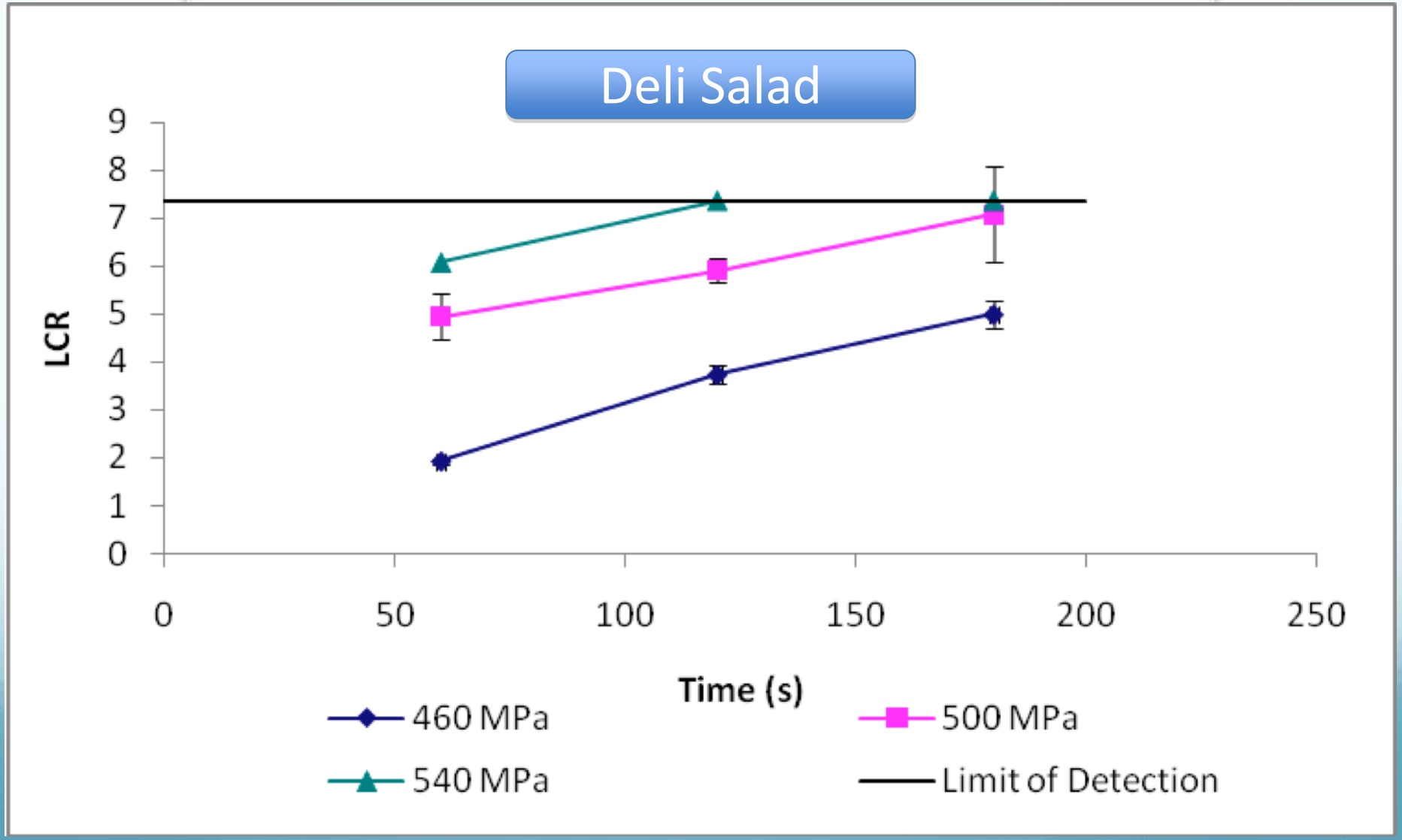
Reversible

200 MPa



Reversible Disassembly of Ribosomes

Inactivation of *Listeria* by HHP



Points to Consider

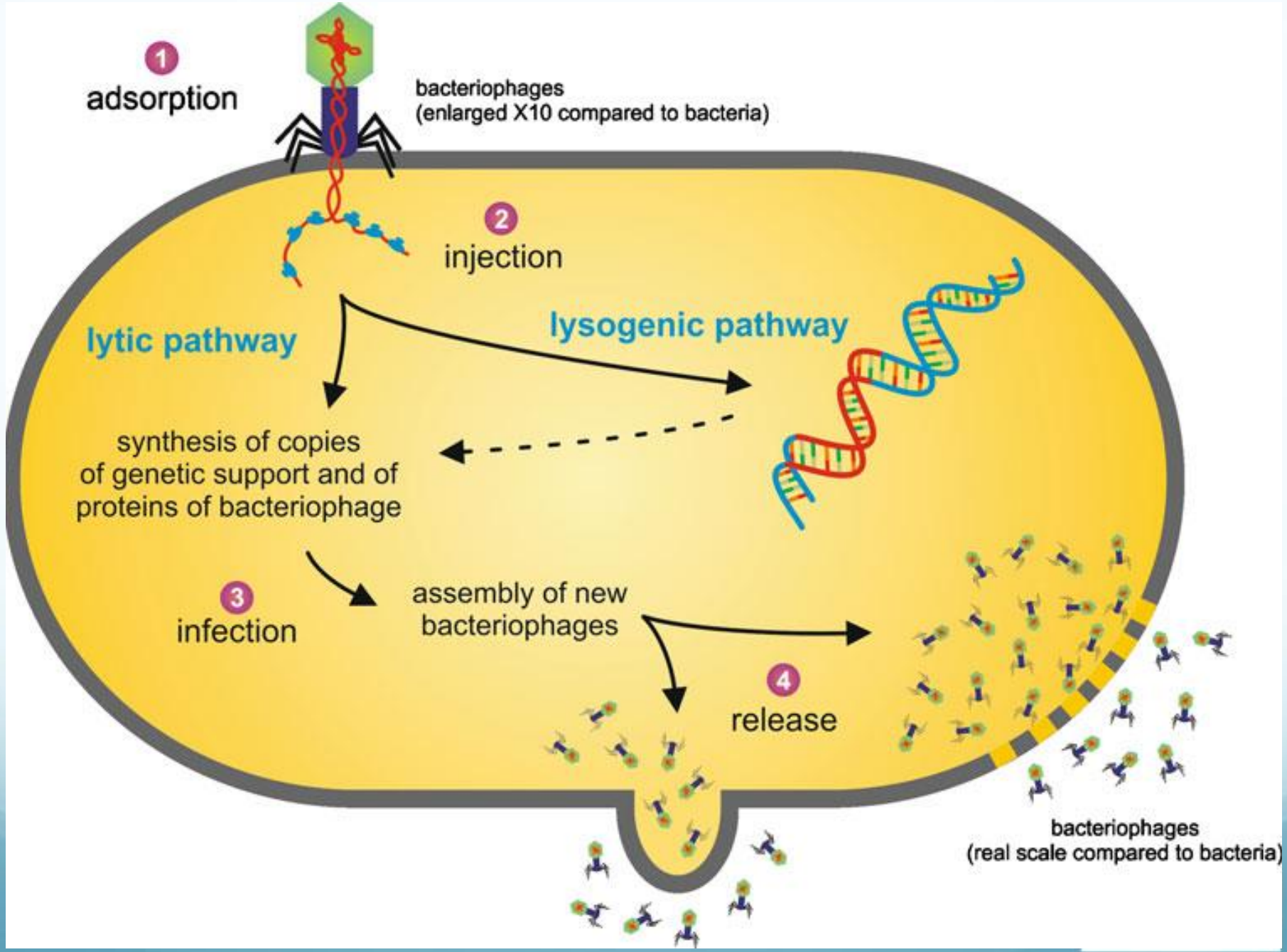
- Low Aw protects cells from HHP treatment
- High acidity enhances HHP
- Used in combination with thermal treatment (Pressure Assisted Thermal Sterilization)
- Tolerance to HHP is strain specific
- Log Count Reduction is product specific
- Little evidence of post-treatment recovery
- Spoilage microbes may recover and hence further antimicrobial hurdle required.
- Endospores activated but not inactivated

Sensory Changes

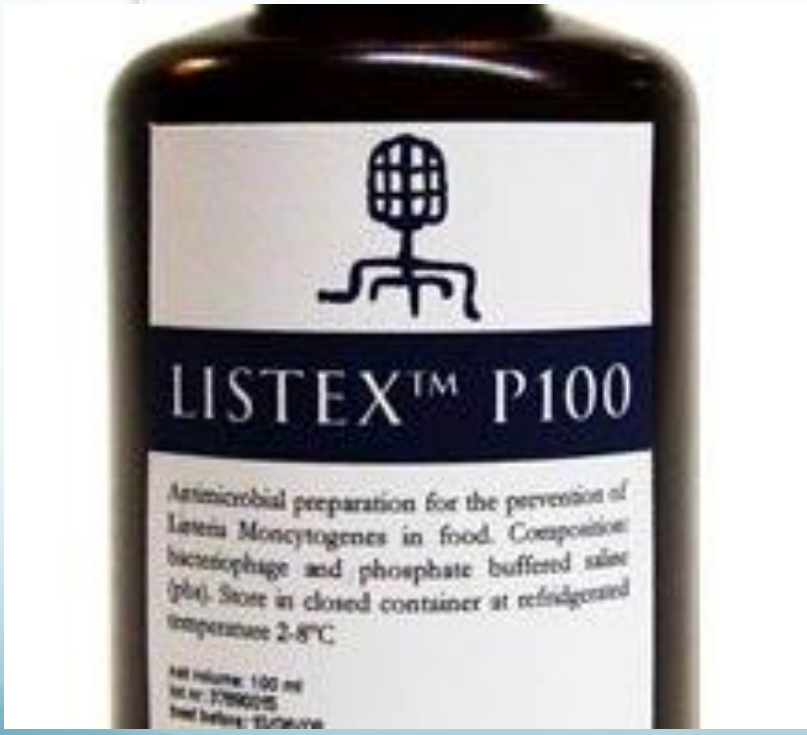
- Coagulation of protein: Adjust pH to neutral
- Approach for producing low salt meat products
- Can be used in combination with modified atmospheric packaging

Biological Control

- Bacteriophages
- Bacteriocins
- Probiotics


1
adsorption
bacteriophages
(enlarged X10 compared to bacteria)
2
injection
lytic pathway
lysogenic pathway
synthesis of copies
of genetic support and of
proteins of bacteriophage
3
infection
assembly of new
bacteriophages
4
release
bacteriophages
(real scale compared to bacteria)

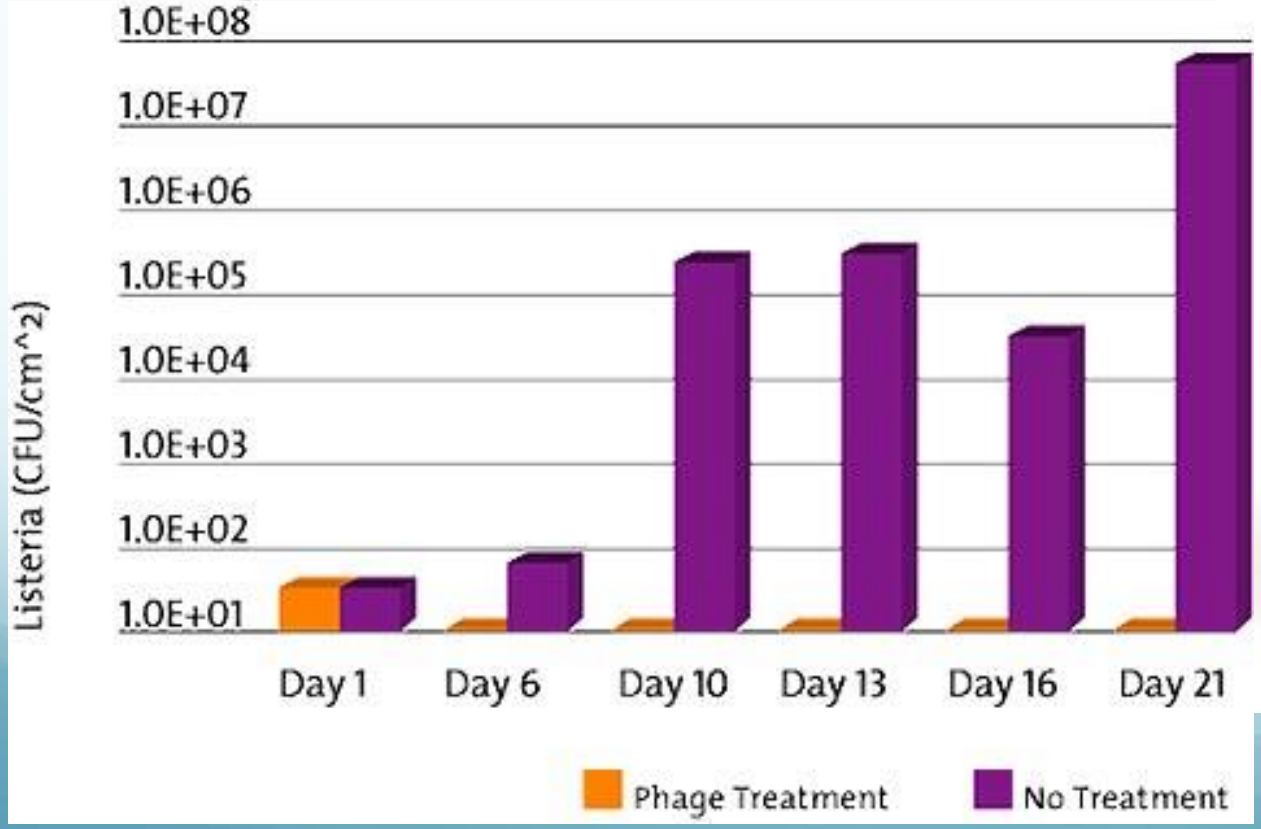
Listex P100



- FDA approved: Processing aid
- Food, Food Contact and Processing Environment
- Phage cocktail with broad host range

Bacteriophage

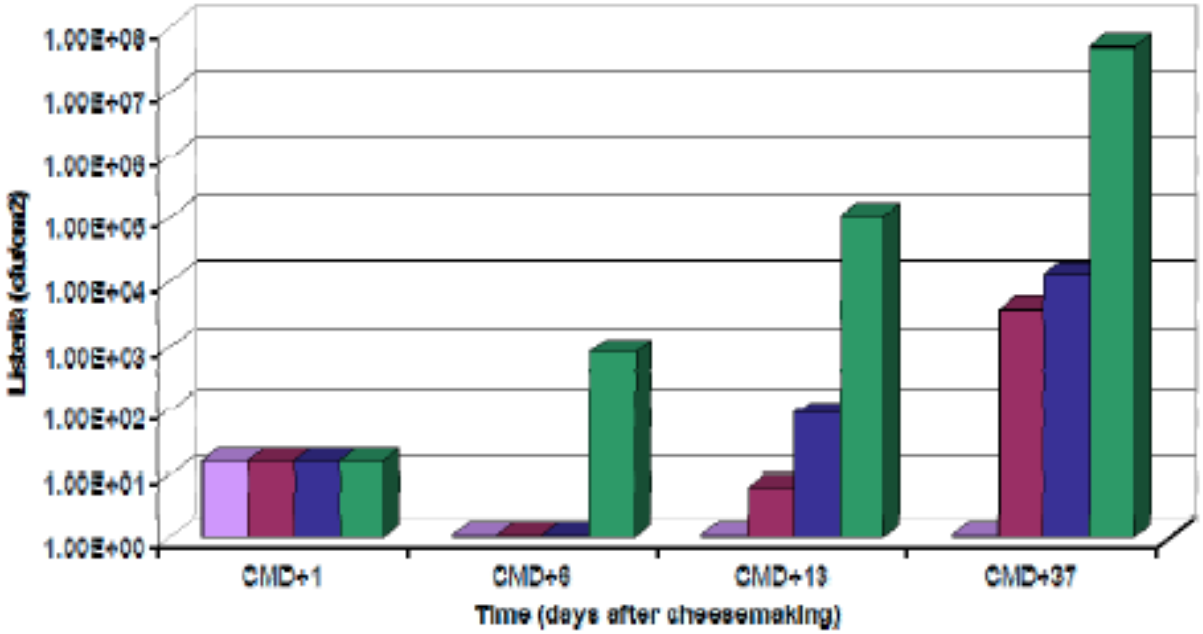
- Listex
- Broad spectrum phage P100



Control of *Listeria monocytogenes* on Food Surfaces

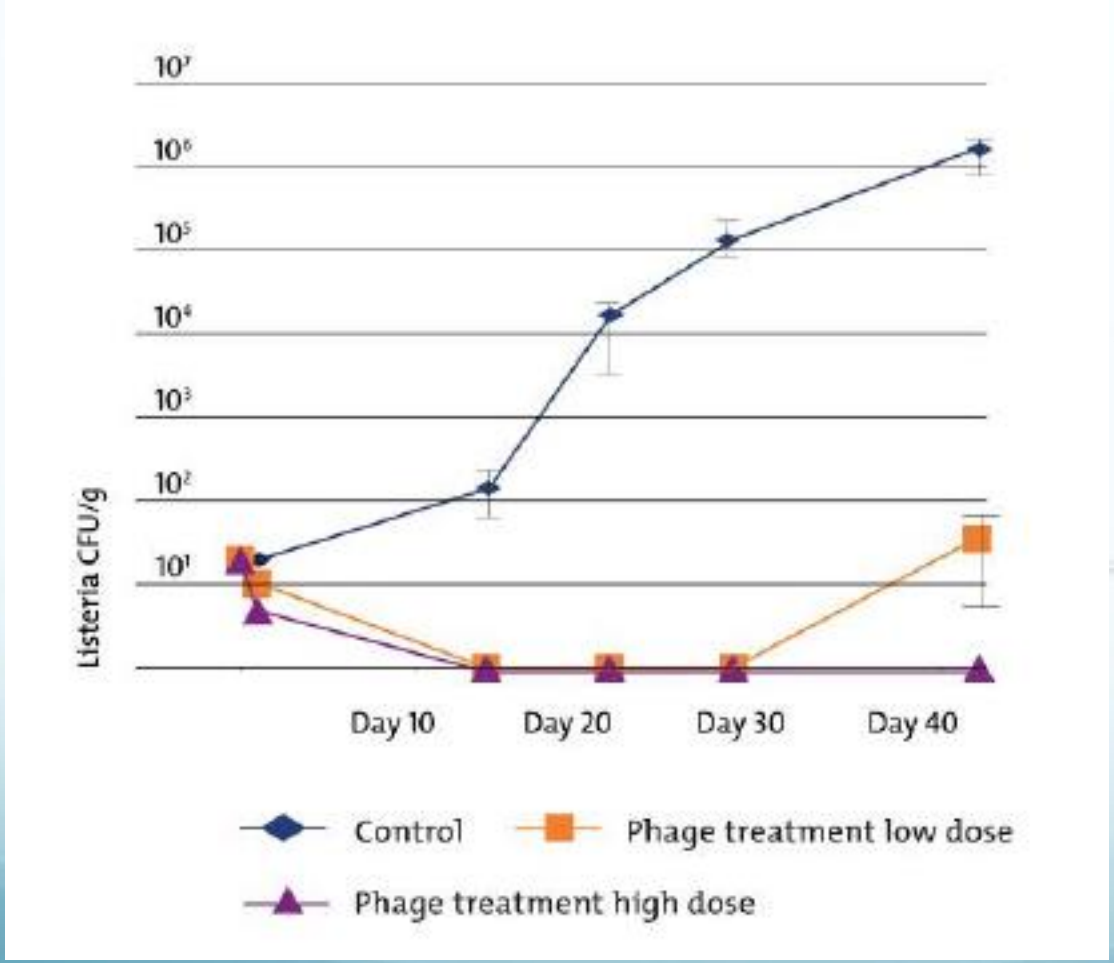
Cheese

•NIZO (Dutch Institute for Dairy Research): Control of *Listeria* in smeared cheeses (Munster)



- Initial contamination: ~20 cfu/cm^2
- Treatments:
  - Frequent high dose
  - Single high dose
  - Frequent low dose
  - Control

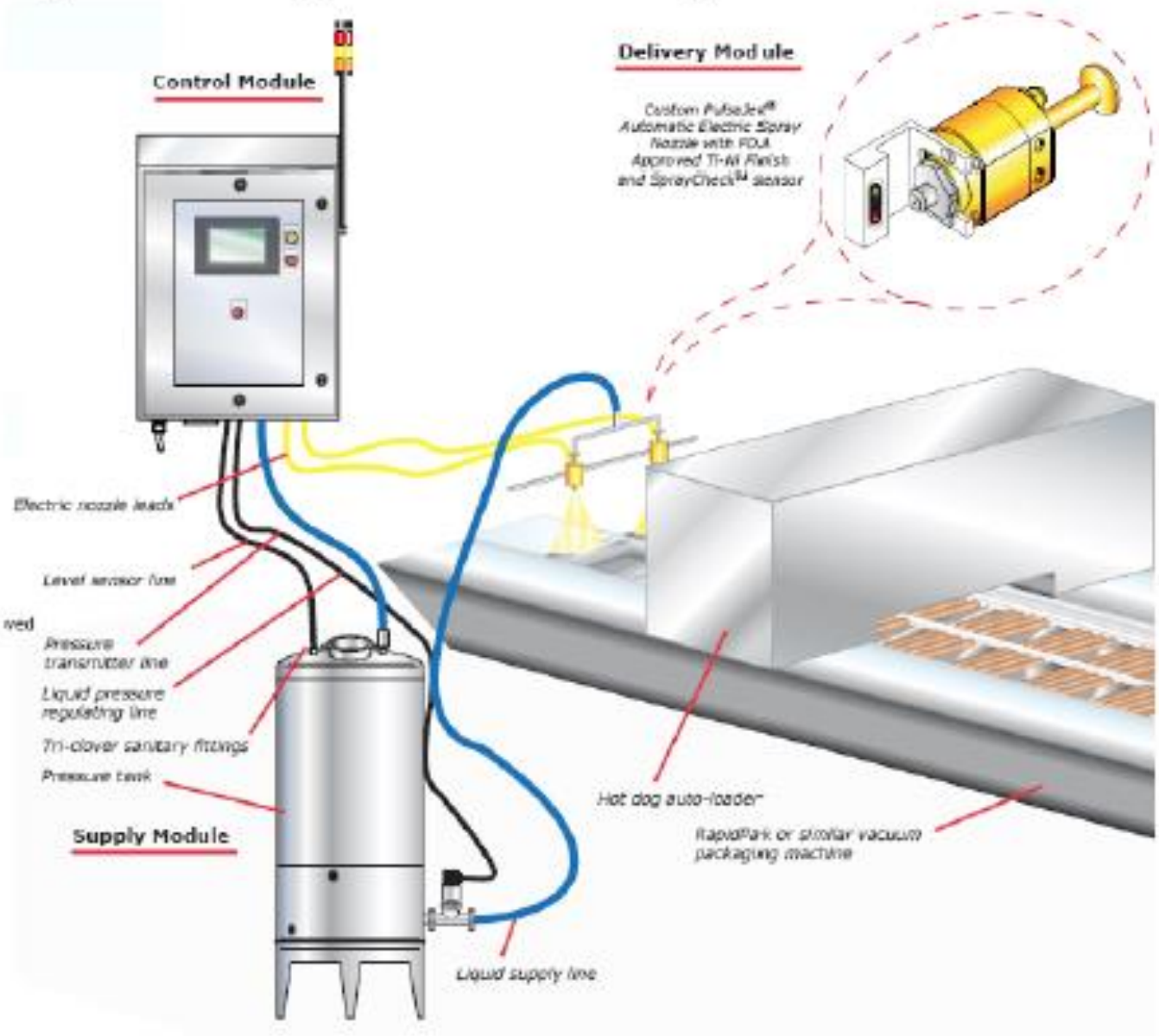
LISTEX Control of Listeria on Ham



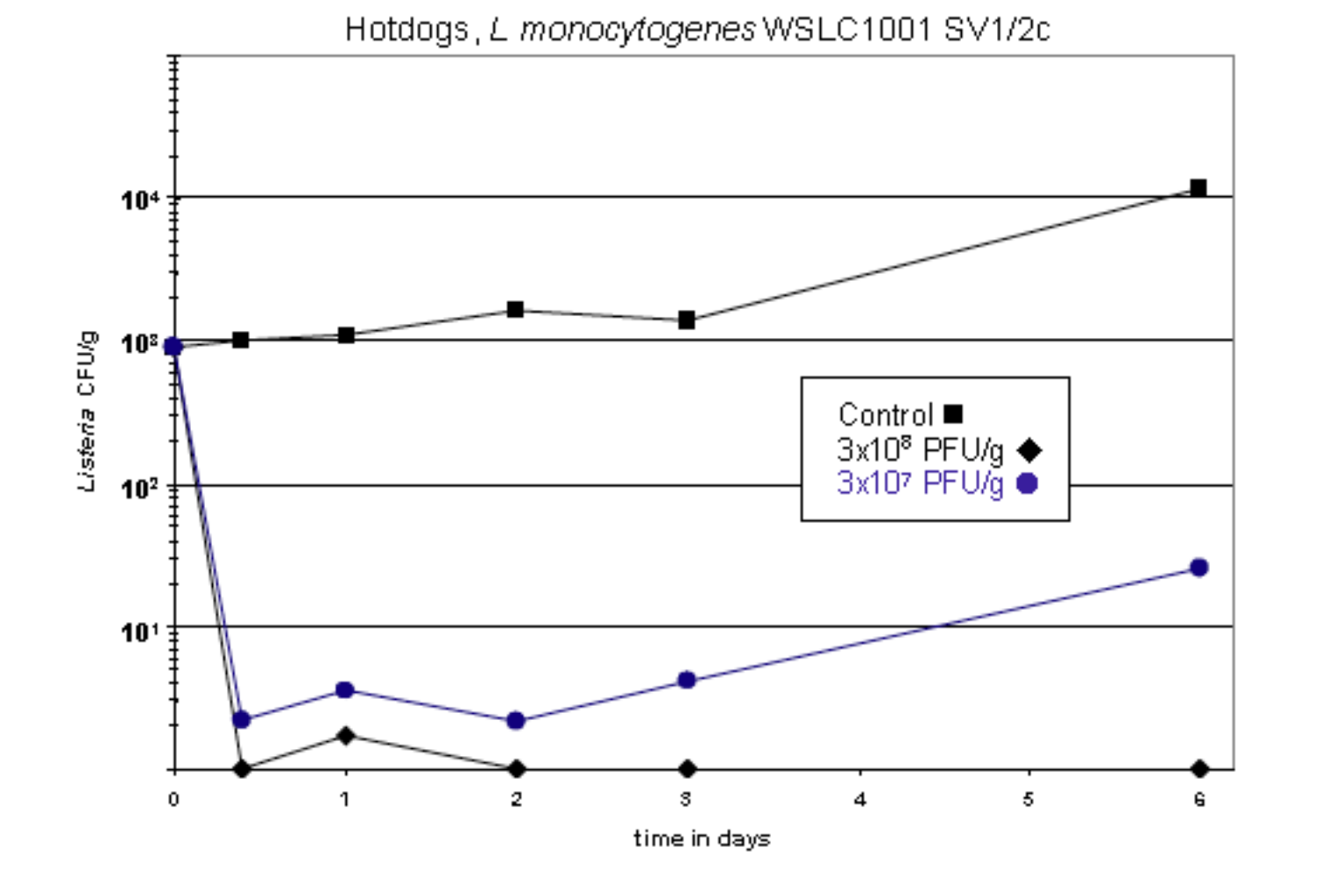
1 x 107 pfu/cm2: Low dose

5 x 107 pfu/cm2: High dose

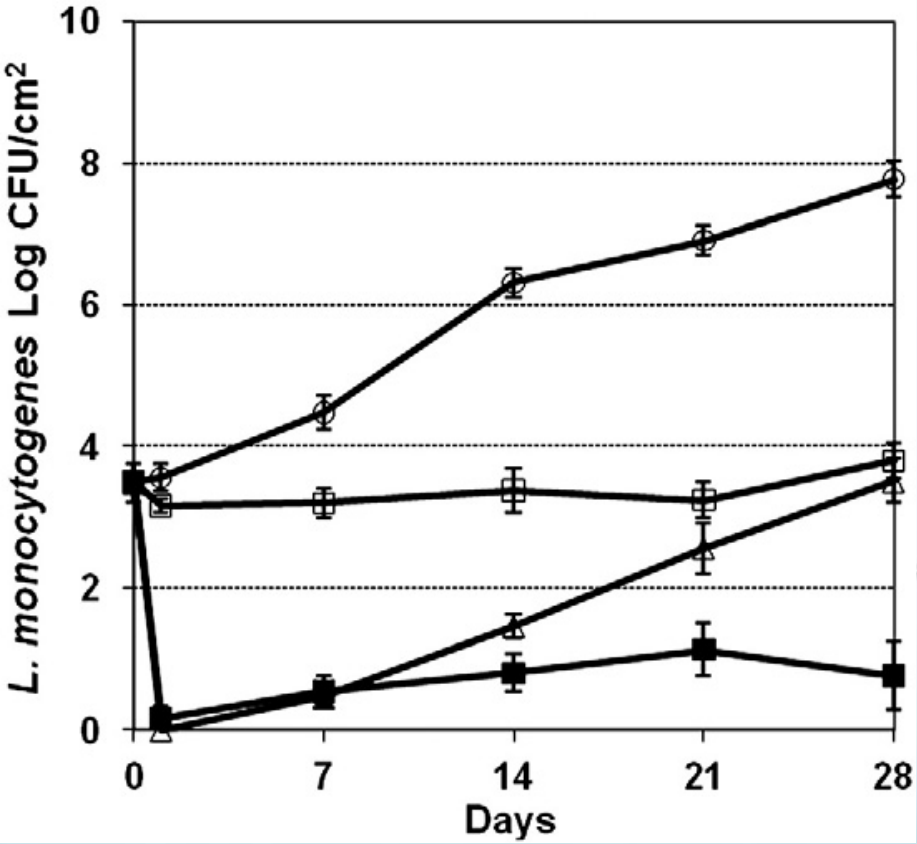
Spraying example: hot dog auto-loader



LISTEX Control of Listeria on Hotdog



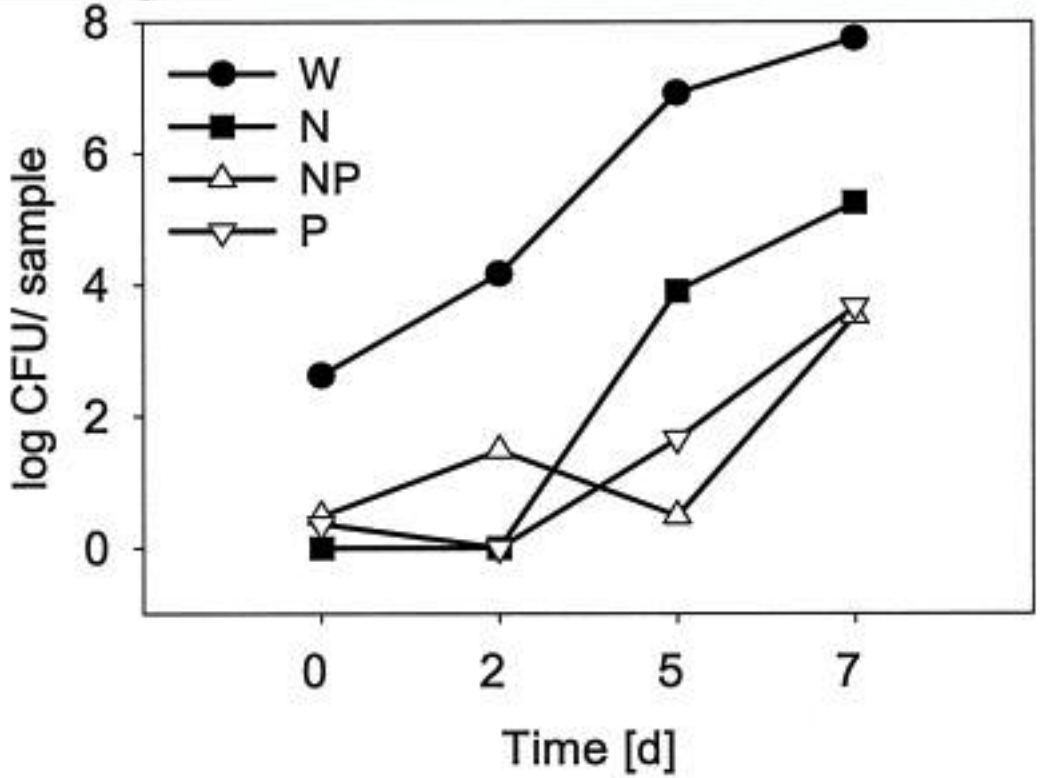
Lactate:Diacetate Cheese



Soni et al., 2012

Fig. 3. Reduction of *Listeria monocytogenes* in QFC after surface treatment with bacteriophage P100 (△) or PL–SD (□) or their combination (■) compared to untreated control (○). The concentration of phage P100 at 10^8 PFU/g and PL–SD (2.8% PL and 0.2% SD) was used for surface application of QFC.

Control of *Listeria* on Watermelon Phages:Bacterocin

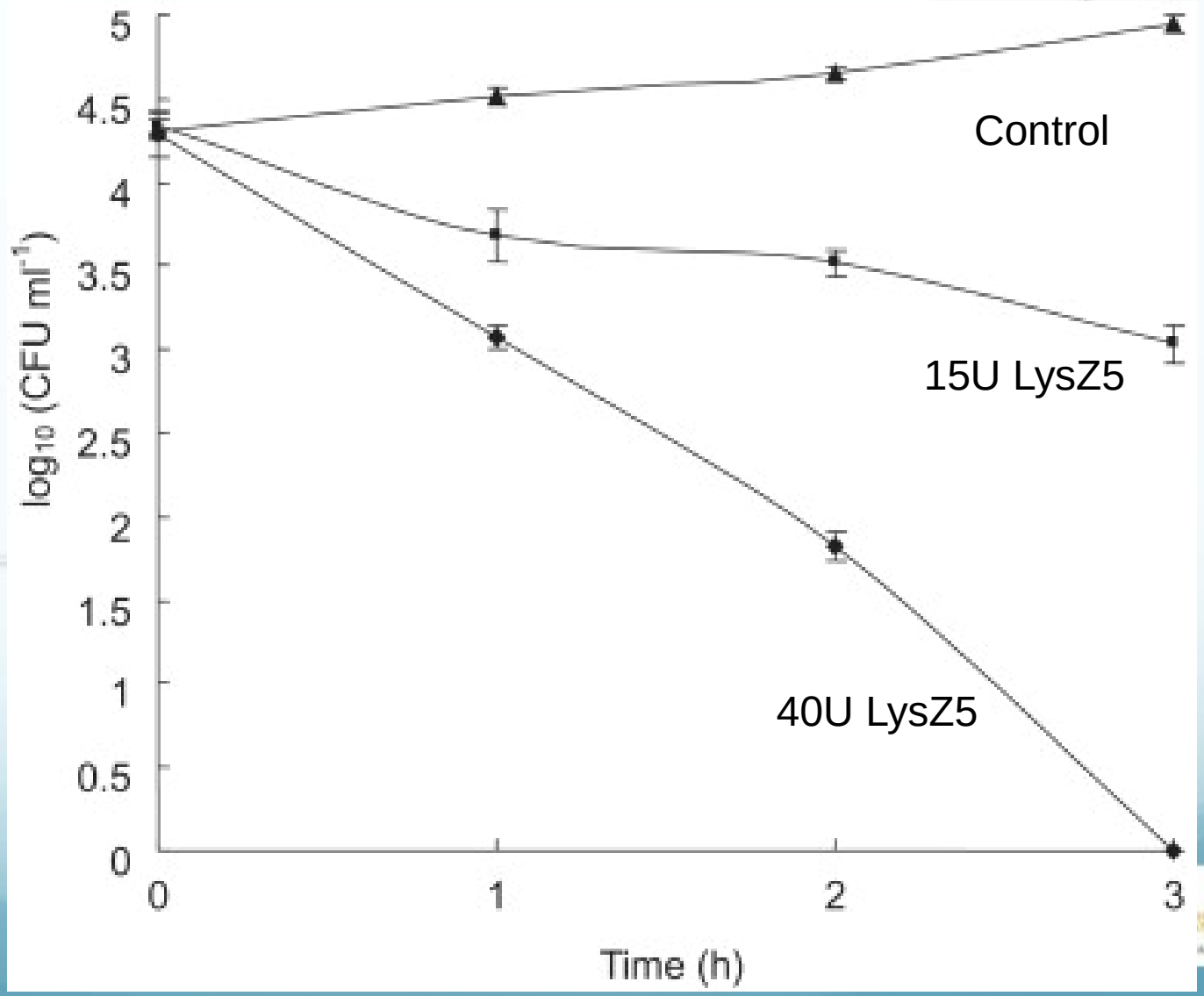


W: Water
N: Nisin
P: Phages

Leverentz et al., 2001

Treatment				
W	2.62	4.61	6.91 a[1] x[2]	7.75 a w
N	0	0	3.90 b x	5.25 b w
NP	0.48	1.48	0.48 c x	3.52 c w
P	0.36	0	1.65 abc w	3.67 c w

Phage Endolysins



Zhang et al., 2012

Developments in *Listeria* Diagnostics

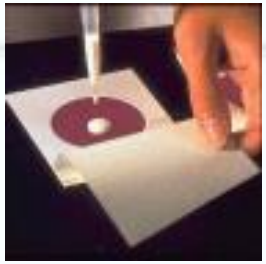
Limitation of Testing

Probability of Missing Contaminated Lot

Number Samples	10%	2%	1%	0.5%
3	73	94	97	99
10	35	82	90	95
60	<0.5	30	55	74
120	<0.5	8.5	30	55
180	<0.5	2.6	16	42
240	<0.5	0.8	9	30

Testing Methods

- Culture



- Immunoassay

- Molecular

PALL GeneDisc

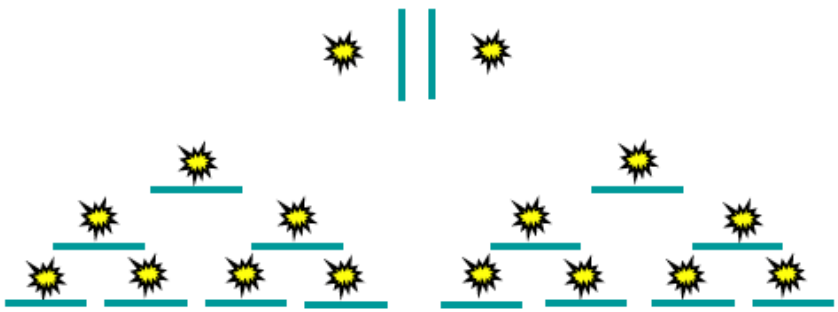
BAX

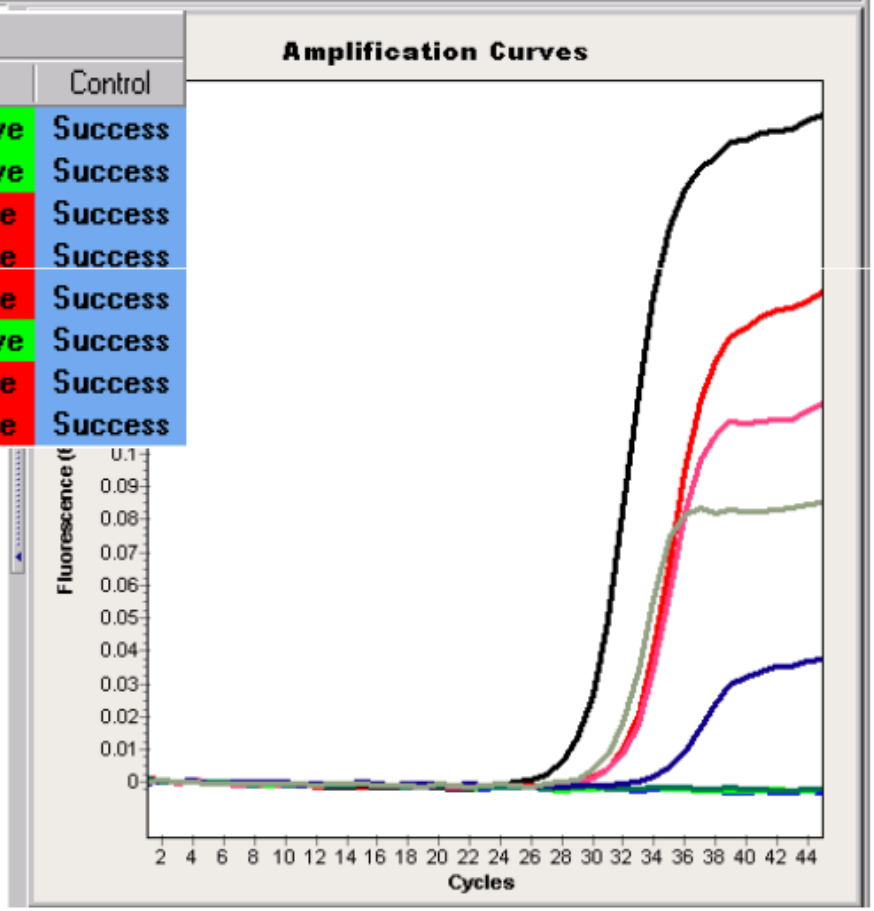
Principle of Real-Time PCR

Qualitative Detection [CC Object from Color Compensation Set (Cat No 2 158 850)]

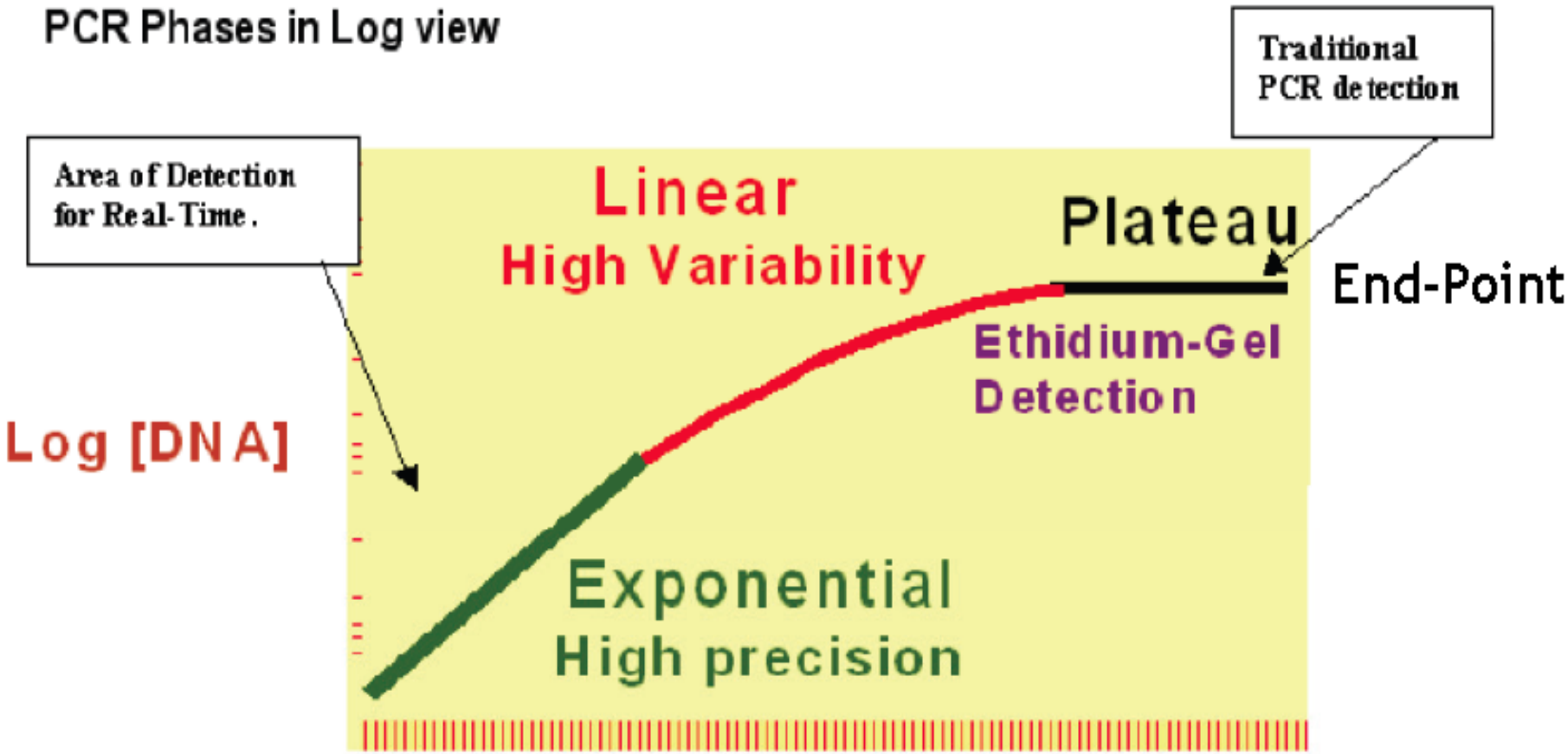
Channel (640/Back 530) ▾ Color Compensation (On) ▾ Program (2) ▾ | Advanced

Samples				Calls		
Include	Color	Pos	Name	Combined	Target	Control
✔		1	no template control	Success	Negative	Success
✔		2	Negative Control	Success	Negative	Success
✔		3	Positive Control	Success	Positive	Success
✔		4	Sample 1	Positive	Positive	Success
✔		5	Sample 2	Positive	Positive	Success
✔		6	Sample 3	Negative	Negative	Success
✔		7	Sample 4	Positive	Positive	Success
✔		8	Sample 5	Positive	Positive	Success





qPCR



No enrichment

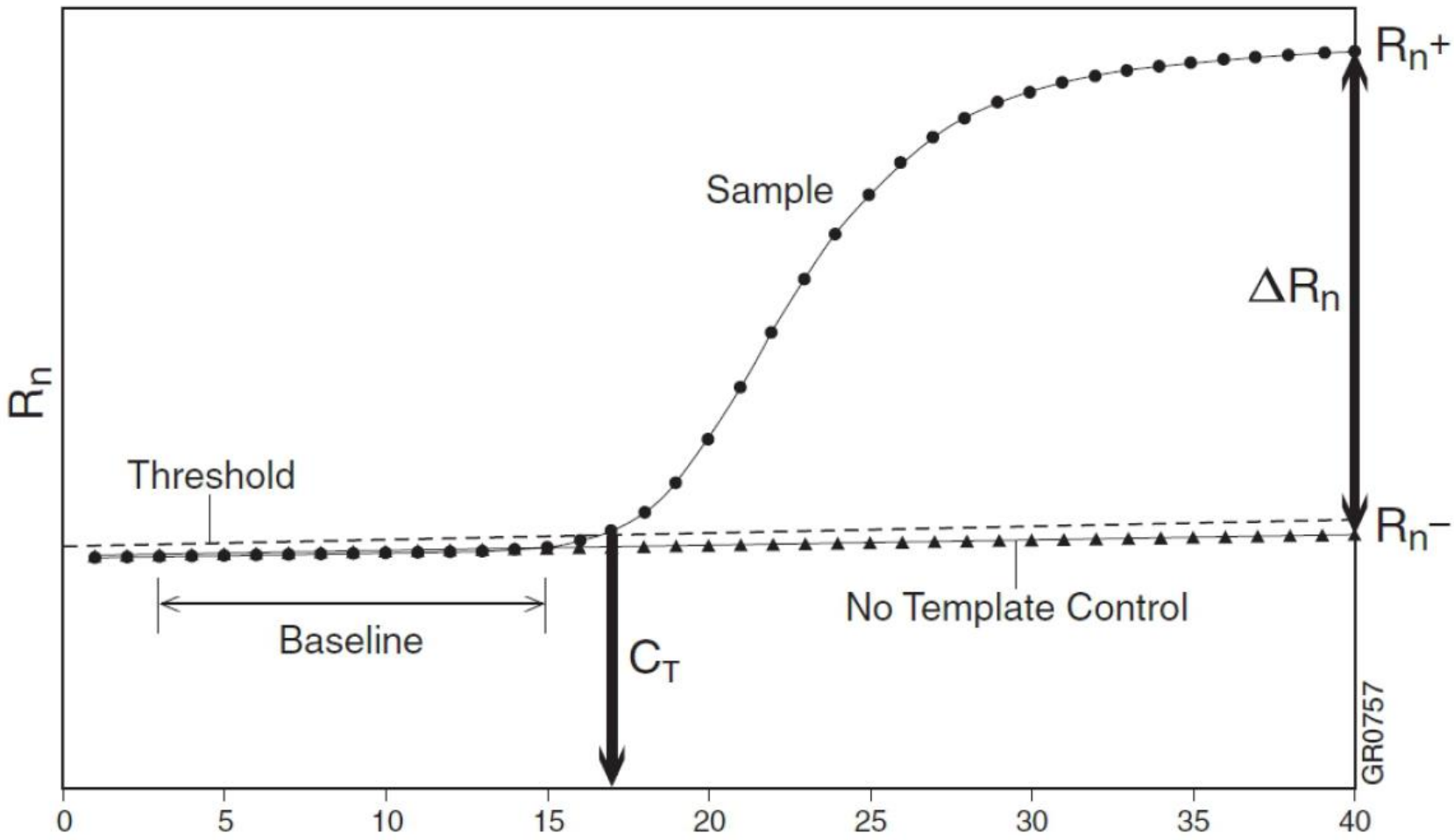
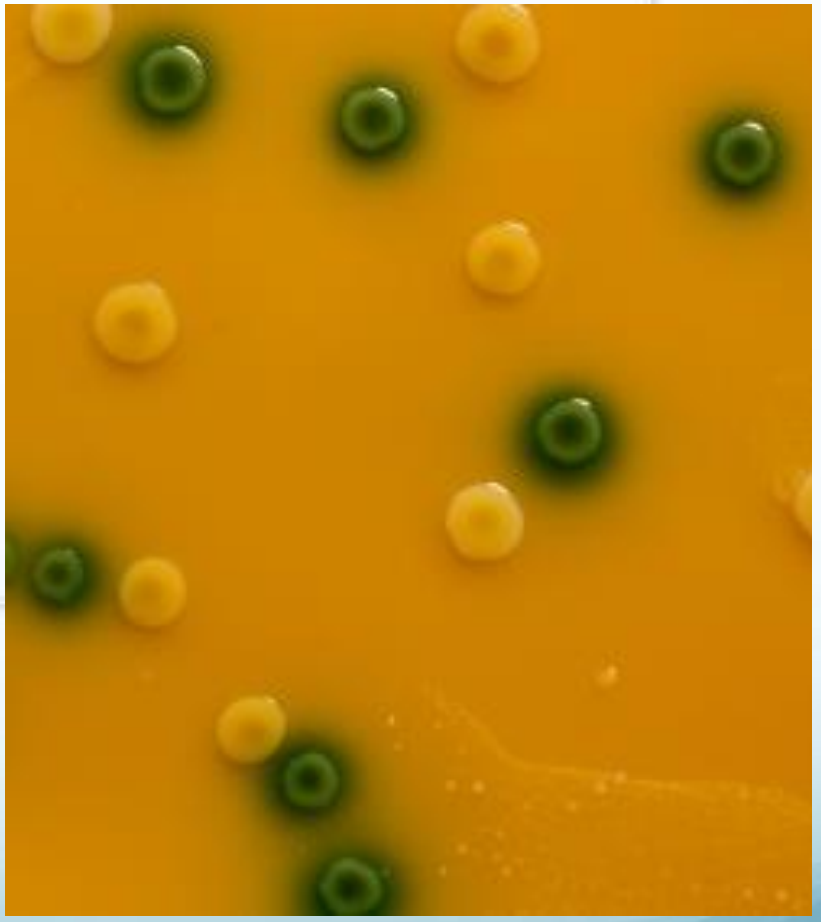


Fig. 5. Demonstration of a TaqMan® amplification curve and parameters. Amplification results in increased fluorescence (Rn). The Ct value reflects the beginning of amplification.

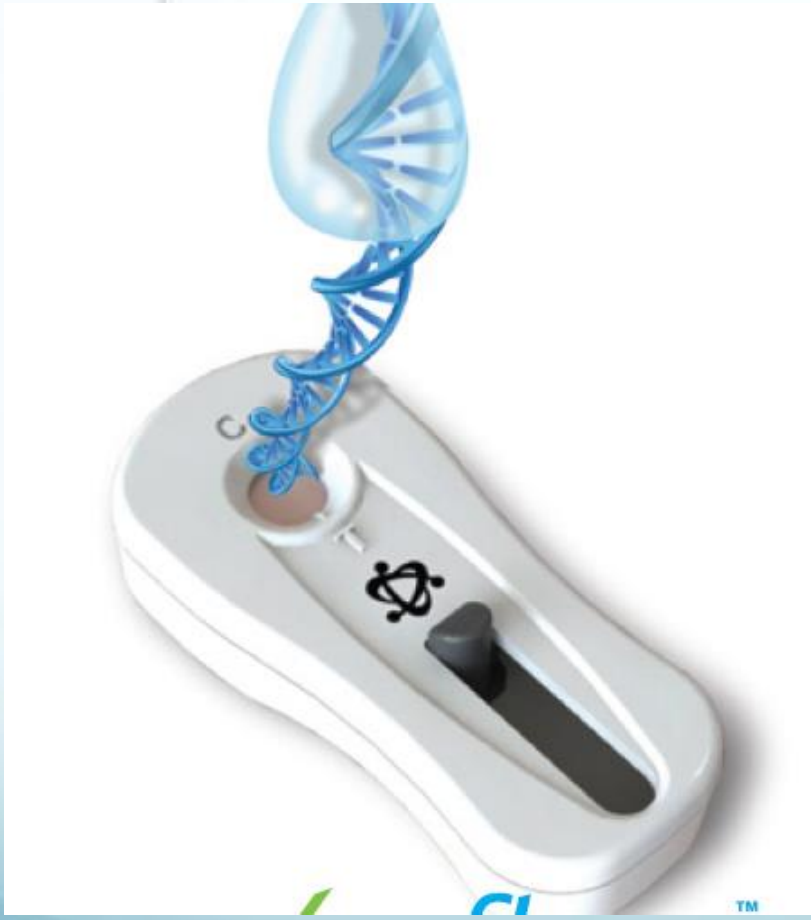
Tebbs et al., 2012

Trends in RT-PCR

- Species identification
- Serotypes
- Automation
- Reduced preparation steps
- Reduced false positives



Veriflow



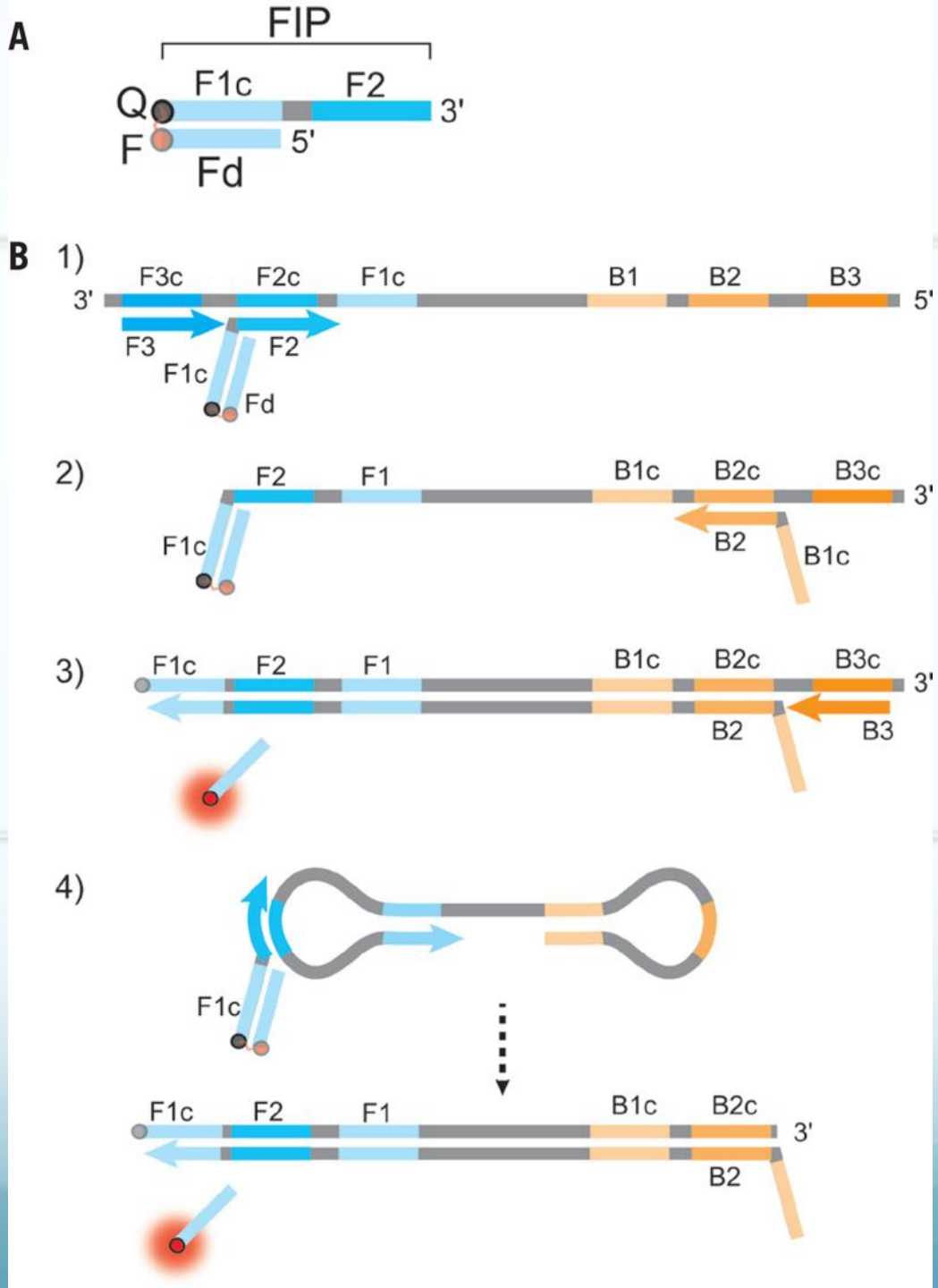
- Enrich culture
- DNA extraction
- PCR reaction
- Add to lateral flow device
- Result within 3 mins

- Low cost and compatible with on-site testing

Isothermal Amplification

- Simplified equipment: No need for thermal cycling
- Adaptable to miniaturization

LAMP


A
FIP
F1c
F2
Q
3'
F
5'
Fd
B
1)
F3c
F2c
F1c
B1
B2
B3
3'
5'
F3
F2
F1c
Fd
2)
F2
F1
B1c
B2c
B3c
3'
F1c
B2
B1c
3)
F1c
F2
F1
B1c
B2c
B3c
3'
B2
B3
4)
F1c
F1c
F2
F1
B1c
B2c
3'
B2

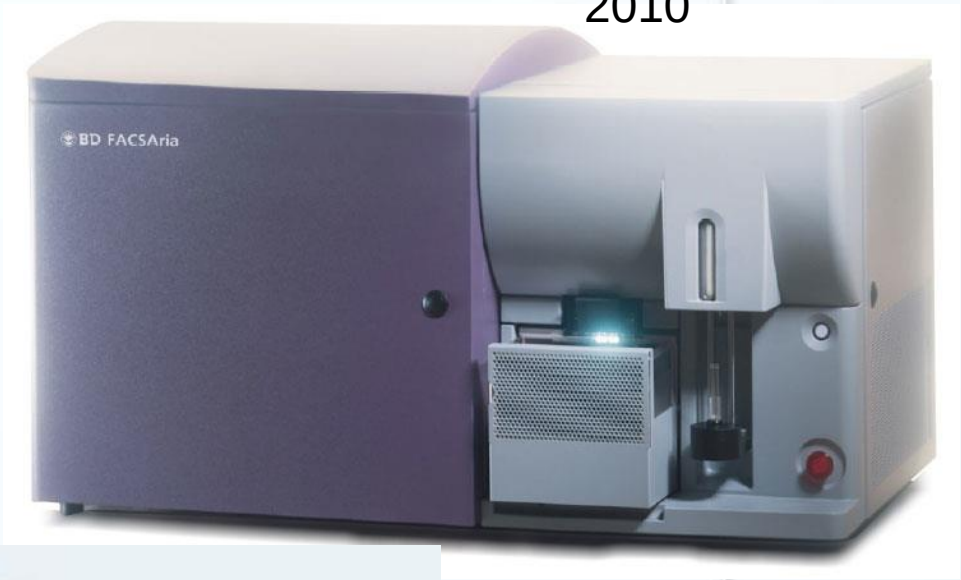

3M
Salmonella
3M
3M
Listeria
3M


Bst
DNA
Polymerase
PPi
PPi
PPi
PPi
APS
ATP-
Sulfurylase
ATP
ATP
ATP
ATP
Luciferase
Light
Light
Light
Light

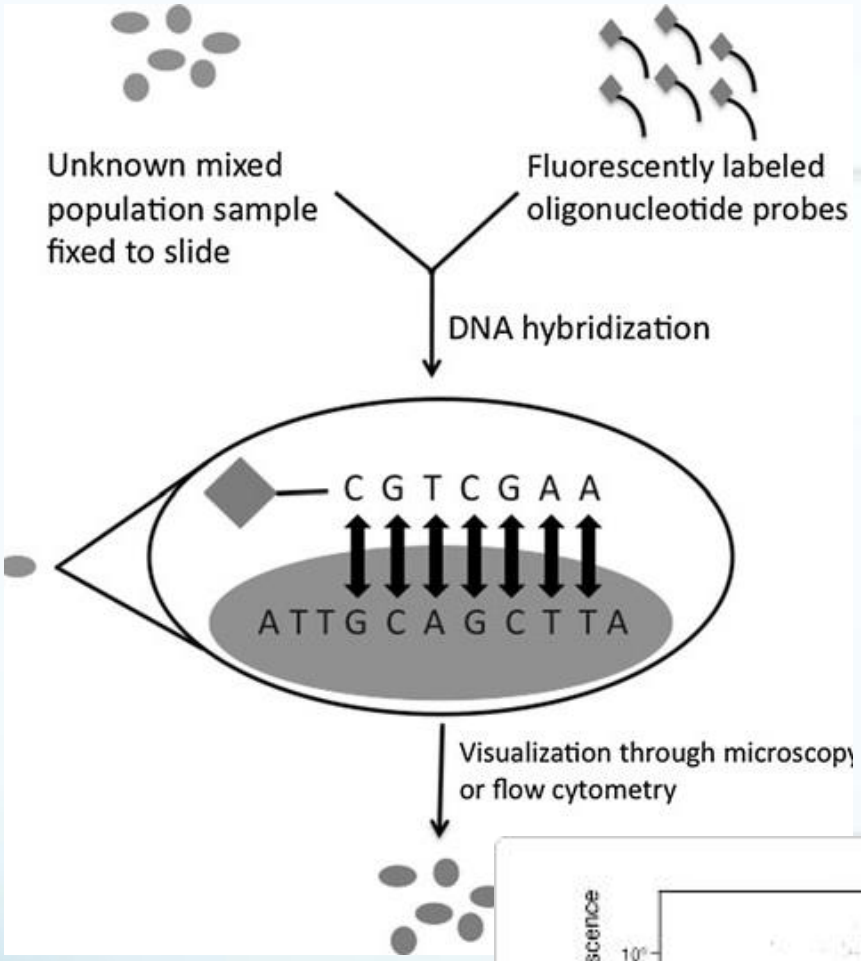
Flow-Cytometry

1999

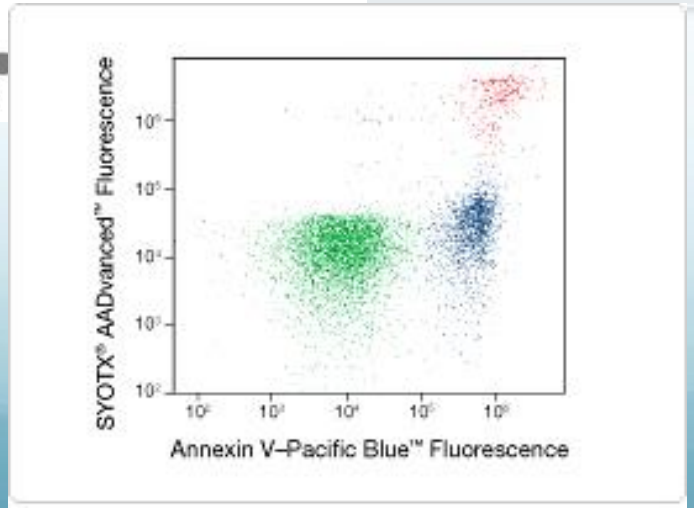
2010



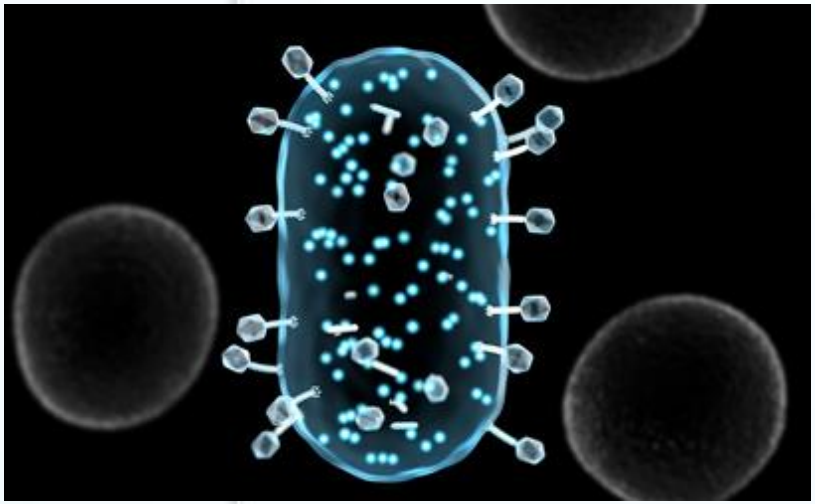
Flow Cytometry



- Real time analysis (2000 cells per min)
- Selective
- Differentiate between viable vs non-viable



Sample6 Diagnostics



- Bacteriophage amplification assay
- Luciferase modified phage
- Detect major Listeria species
- Culture free; Rapid
- AOAC approved

PathoGenetix's Genome Sequence Scanning

- 10 million bases per second
- 5 h assay (40 samples per 24 h)
- No specific target pathogen
- Current being evaluated by CDC ($40m project)

Conclusions

- Initiatives have decreased the incidence of *Listeria*.
- Greater susceptible populations
- Control at retail and consumer interface
- Policy to focus on high risk products
- Advances in diagnostics for rapid on-site detection