



**EBI FOOD SAFETY**

**LISTEX™**

**NATURE'S SOLUTION FOR LISTERIA**

**2009**



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EBI FOOD SAFETY

# Introduction to EBI Food Safety





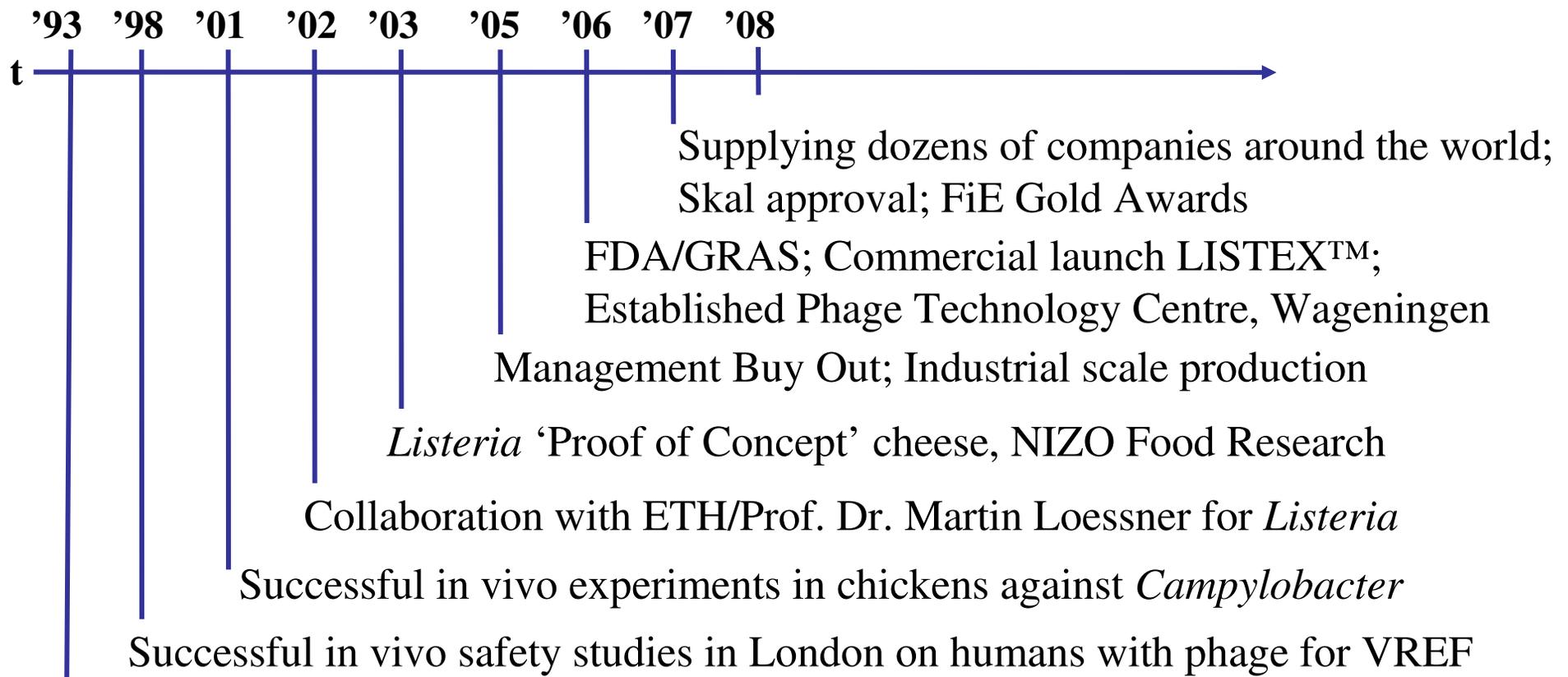
## EBI Food Safety

- Fast growing Dutch company
- Research based
- Cooperation with major universities and institutes
- Excellent lab facilities
- Flexible production
- Located in The Netherlands, Food Valley





# EBI Food Safety, from pharma to food



EBI established, spin out of National Institute of Health (NIH)



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# Foodborne Pathogens





# Real Events Happening Daily to Real People

US alone: 76 million cases of foodborne illness annually  
 EU 2006 +9% listeriosis cases (1583 cases); 30% mortality

Organism	Case-fatality ratio
<i>Listeria</i>	<b>0.2</b>
<i>Clostridium b.</i>	0.08
<i>Brucella spp</i>	0.06
<i>E. coli O157:H7</i>	0.009
<i>Salmonella</i>	0.007

Organism	Costs per case
<i>Staph. Aureus</i>	€ 581
<i>Salmonella</i>	€ 700
<i>Campylobacter</i>	€ 786
<i>Clostridium</i>	€ 5.013
<i>E.coli O157</i>	€ 23.318
<i>Listeria</i>	<b>€ 99.863</b>

Source: Food related illness and death in the US [www.cdc.gov/ncidod/eid/vol5no5/mead.htm](http://www.cdc.gov/ncidod/eid/vol5no5/mead.htm)





# Besides the victims companies are hit hard by recalls and bad press

## FINANCIAL POST

### Listeria recall costs Maple Leaf Foods Millions

Canada, October – 29 2008

Maple Leaf Foods Inc. on Wednesday posted a third quarter loss of \$12.9-million due to the financial impact of this year's tainted-meat recall following a deadly outbreak of listeriosis food poisoning. So far 20 people died in the nation-wide recall.

The Toronto-based company, one of Canada's largest food processors, said earnings per share amounted to 10 cents, compared with \$1.67 a share a year earlier when it recorded a profit of \$220.4-million

## BELFAST TELEGRAPH

### Three patients die as Listeria outbreak hits the Royal Belfast, June - 19, 2008

Northern Ireland's biggest hospital was at the centre of an investigation last night after the deaths of three patients who contracted the Listeria bug. Dr Stevens said that while investigations are ongoing, the hospital could not rule out that the patients fell ill after eating a hospital meal.

## The New York Times

### Nationwide recall issued for 286,000 lbs of prepared meat products

New York, May - 4, 2008

New York City company that sells processed foods has recalled 286,000 pounds of prepared beef, pork and poultry. The danger is from the bacterium Listeria, which causes Listeriosis, according to the U.S. Department of Agriculture.

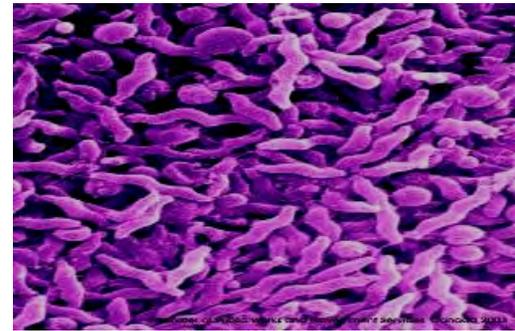


# Foodborne pathogens must be addressed

*Listeria* (USD 2.3 billion)



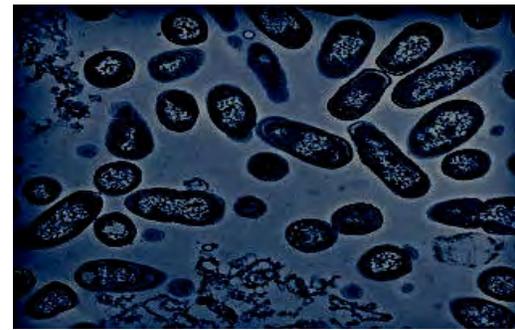
*Salmonella* (USD 2.4 billion)



*E.Coli* (USD 1 billion)



*Campylobacter* (USD 1.2 billion)

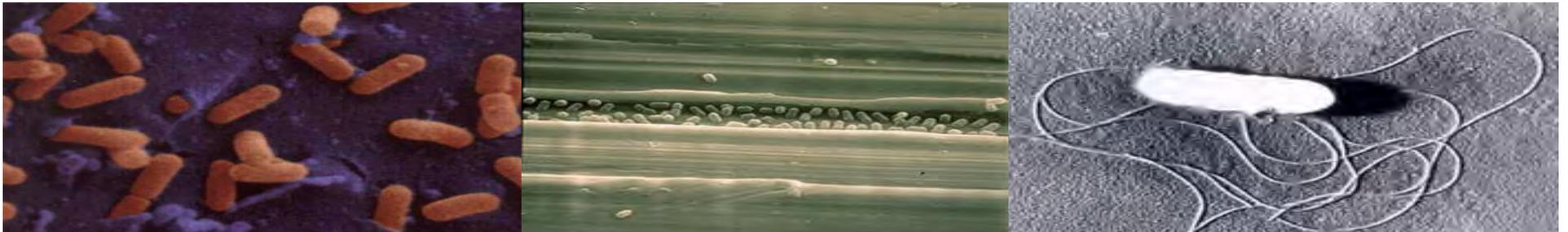


Source: USDA  
Economic Research Service 2000



## *Listeria monocytogenes*

- Ubiquitous (also in modern processing equipment)
- Hardy: grows at refrigeration temperatures (0-4°C) and in low oxygen conditions (i.e. packaged food)
- Minimum pH is 4.4, salt tolerant, pasteurization will kill *Listeria*
- Pathogenic, causes listeriosis (**mortality: ~25-30%**)
- Variable incubation (up to 2 months after exposure)
- Incidence rising despite rigorous and effective control measures
- Zero-tolerance policy in US, Aus/NZ and Russia



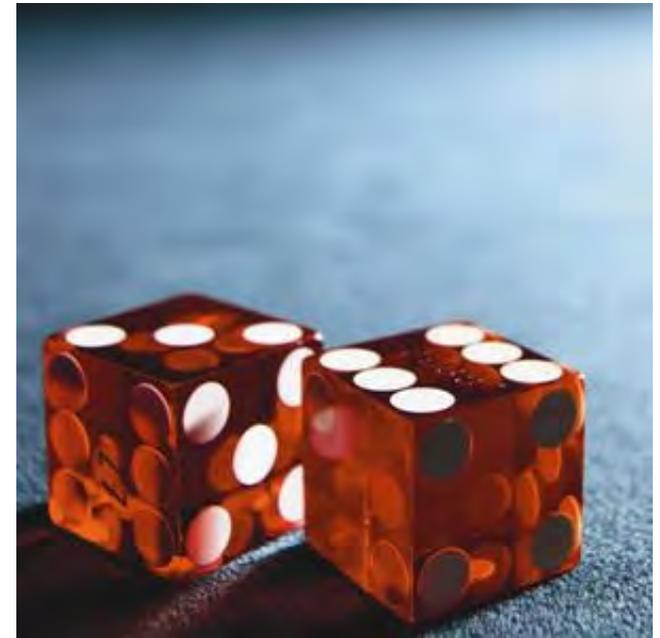


**“Food Safety is one area of your operations where you bet your business each and every day.”**

Dr. David Theno

Editor Meat&Poultry Magazine

Retired Chief Product-Safety Officer Jack in the Box Inc. during 16 years. Credited with revolutionizing food safety in the food chain.





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# Bacteriophages





**They were here yesterday**

**They will be there tomorrow**

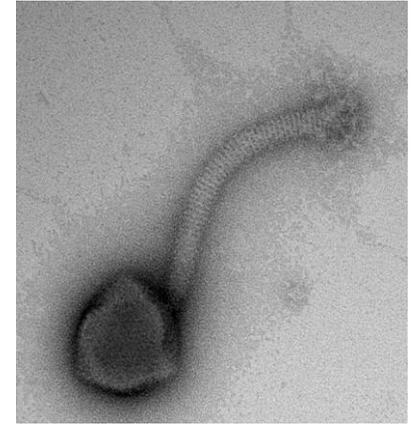
**We can use them today**





## Bacteriophages (1)

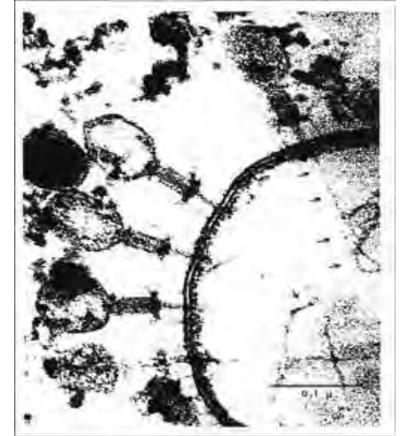
- Natural enemy of bacteria
- Greek “for bacteria eater”
- Most abundant micro-organisms on earth ( $>10^{30}$ )
- $10^7 - 10^9$  phages can be found in 1 ml of seawater
- Bacteriophages are abundant in foods that have little processing
- Common residents in the intestinal tract



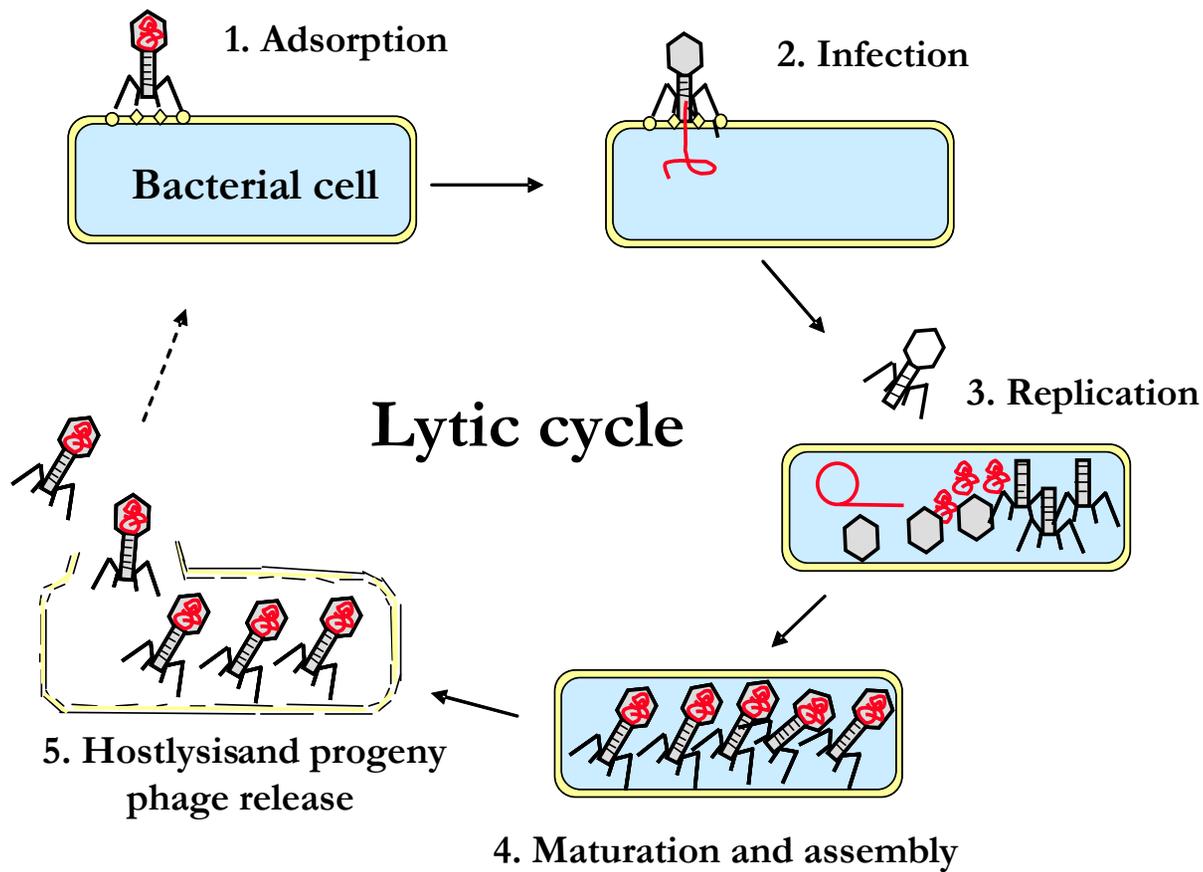
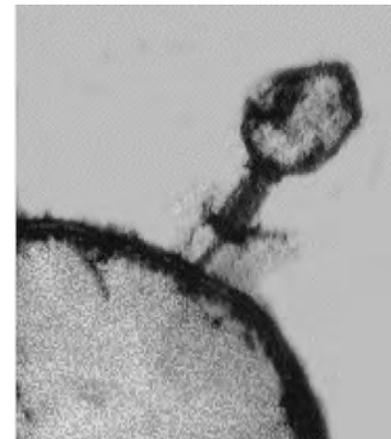


## Bacteriophages (2)

- Typically 100 times smaller than bacteria
- Harmless to humans animals and plants
- Affect only bacteria
- Mostly ‘species-specific’, and mostly specific *within* a species
- Do not affect the properties of treated food
- Leave no ecological footprint (composed of proteins and DNA, eventual breakdown products: amino acids and nucleic acids)



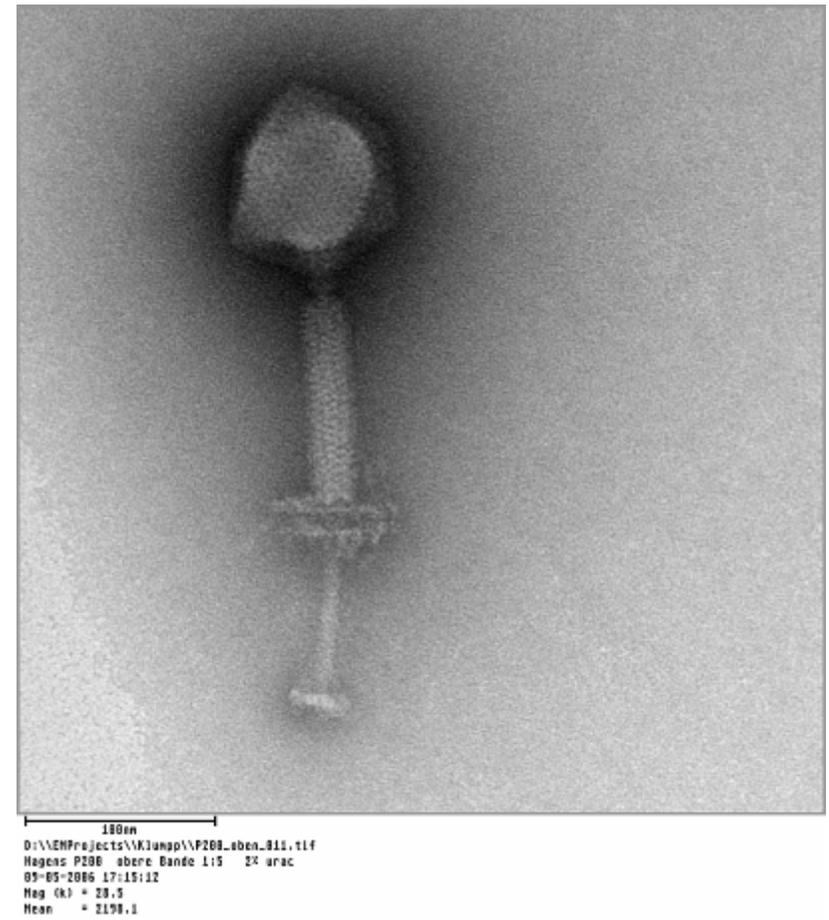
# Bacteriophages (3)





## LISTEX™ P100

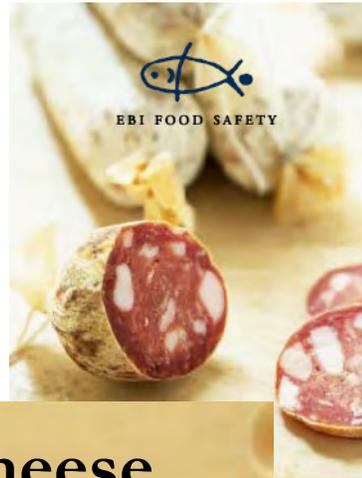
- LISTEX™ infects ~99% of *Listeria monocytogenes*, thousands of isolates tested
- Generally Recognized As Safe (GRAS)
- Now applied successfully across the globe





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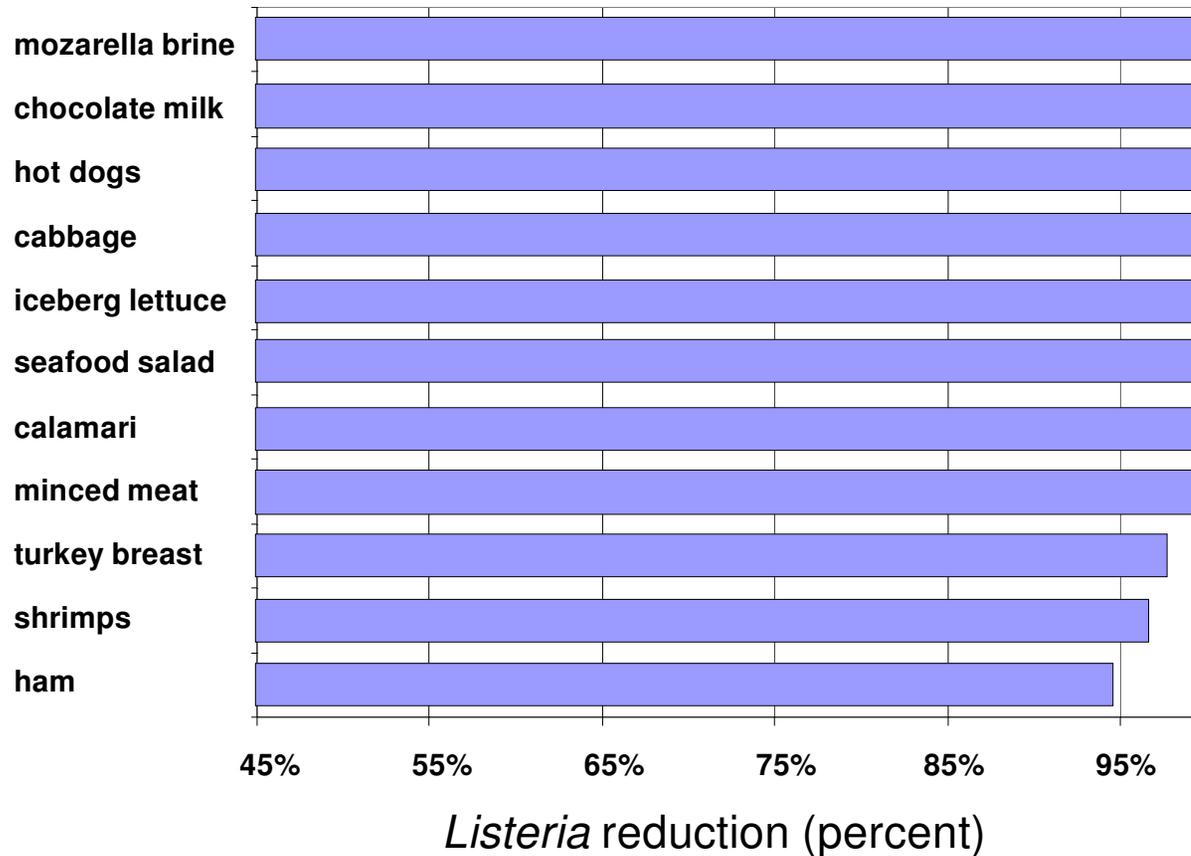
# Applications





# General efficacy in foods

Data generated by ETH (Swiss Federal Institute of Technology Zurich): Efficacy of LISTEX™ for control of *Listeria* in different foods (after storage for 6 days at 6°C).





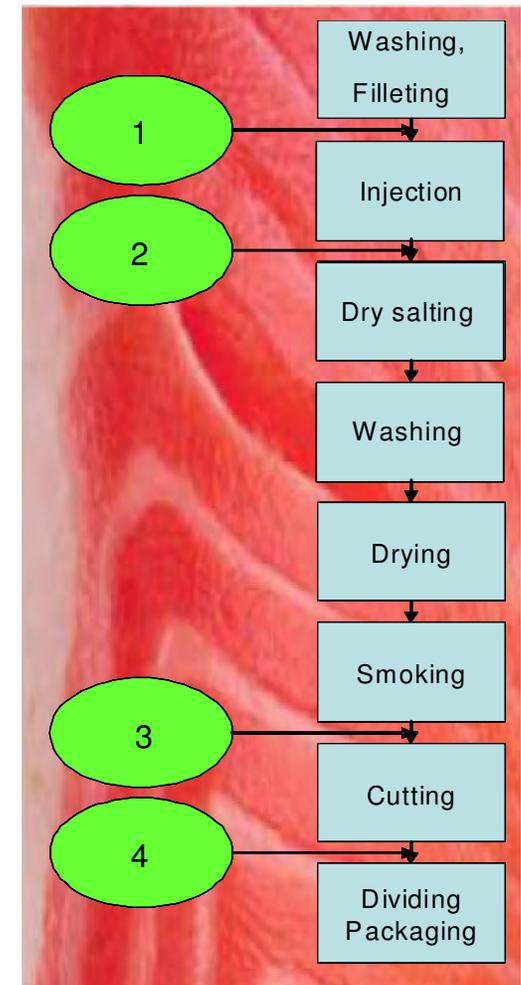
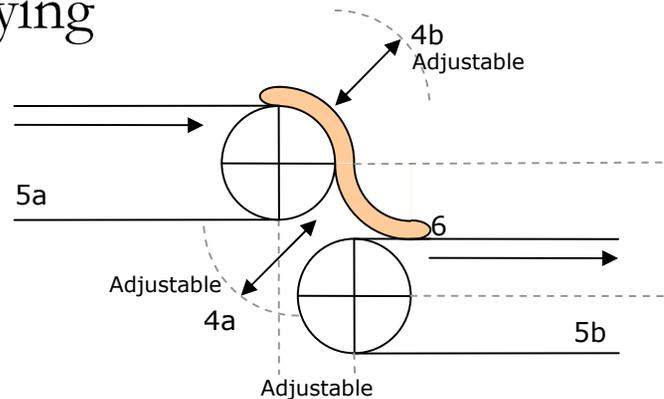
# LISTEX™ controls *Listeria* in fish processing

## Situation:

- Farmed fish is often contaminated
- Cold smoking and salting will not fully remove *Listeria*

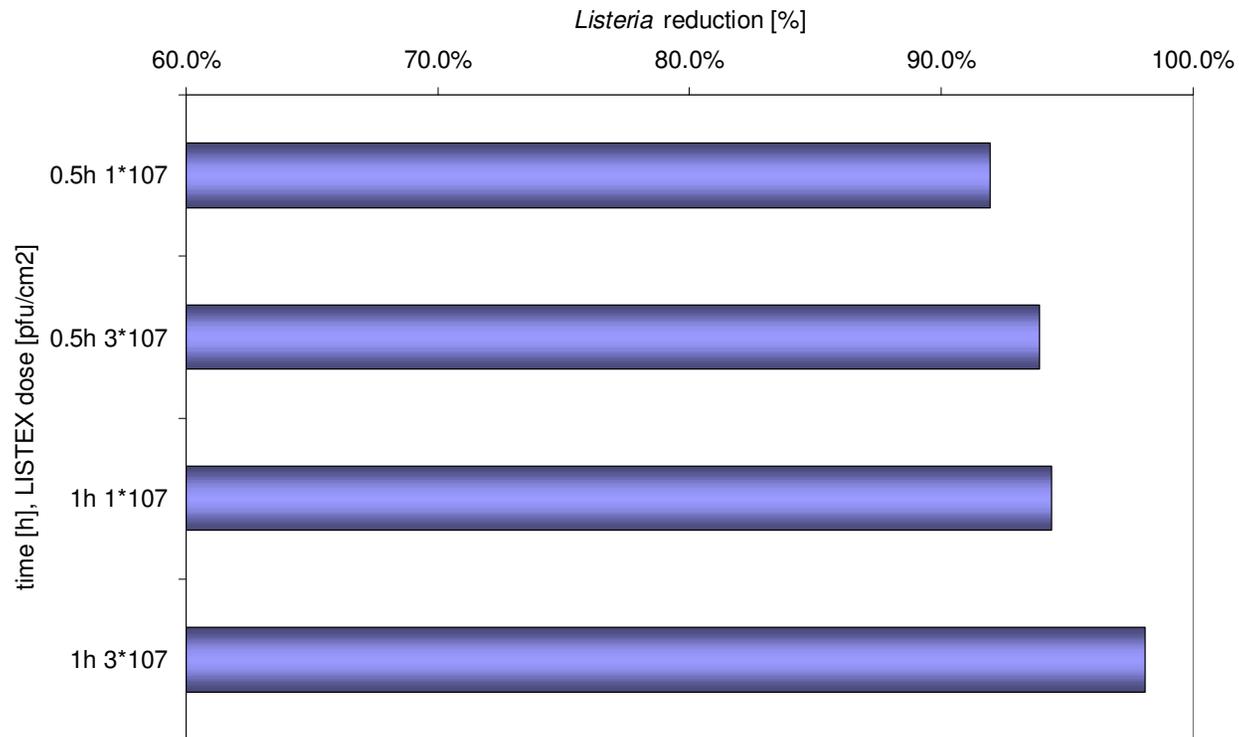
## • Application:

- Raw fillets: prevents transfer in facilities
- Smoked fish: kills after smoking
- Dipping or spraying



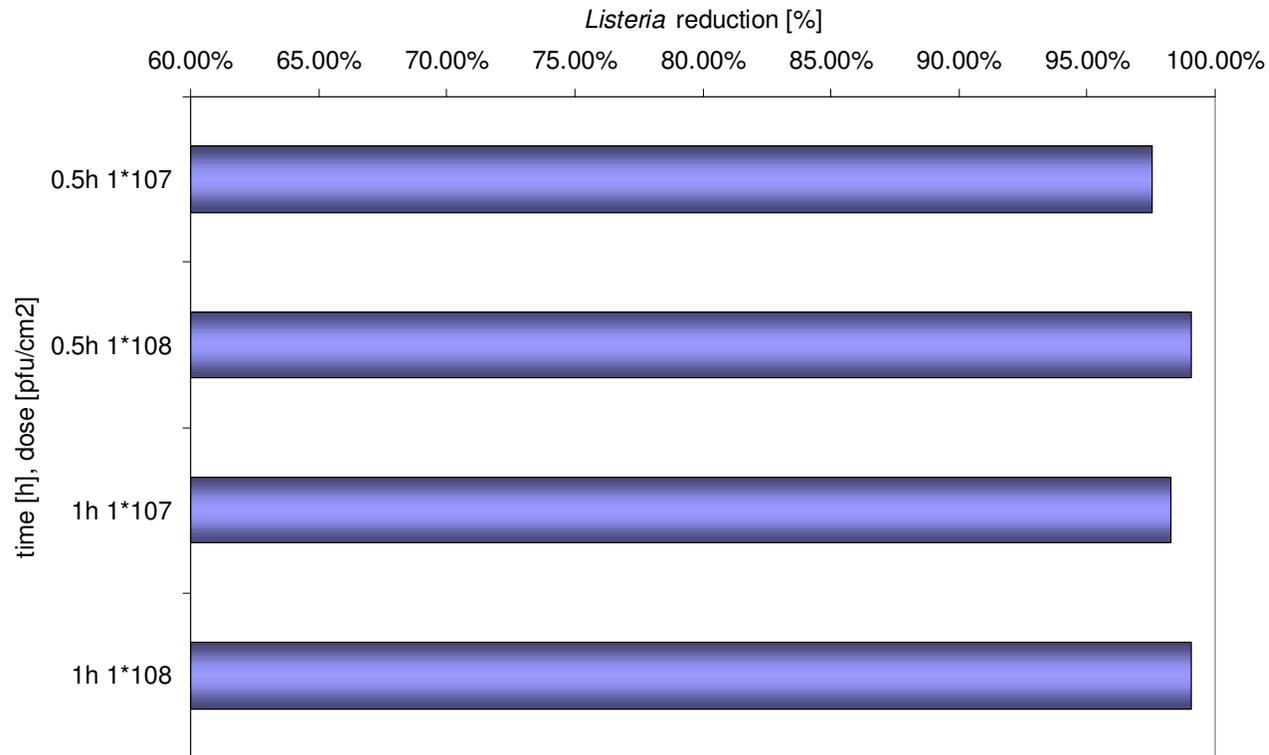


# Significant reductions with LISTEX on raw fish



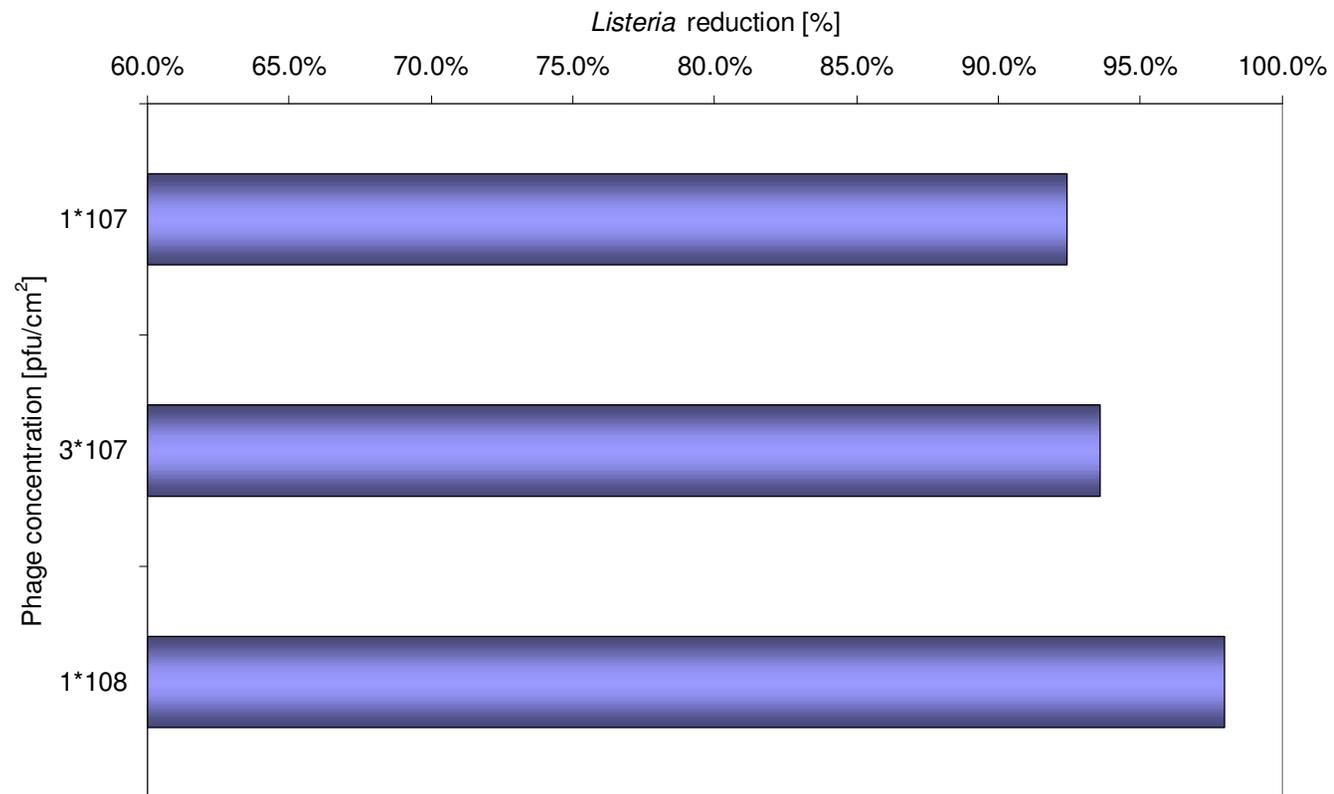


# *Listeria* reduction with LISTEX improves after salting of fillets





# Treatment of salmon after smoking as alternative application point





## Fish – application example

To treat 200 salmon fillets:

- Calculate surface of fillets  
(e.g. 1900 cm<sup>2</sup>/fillet, 2 kg/fillet)
- Advised dosage :  $5 \times 10^7$  pfu\*/cm<sup>2</sup>
- Dosage : 1 bottle for 400 kg fish

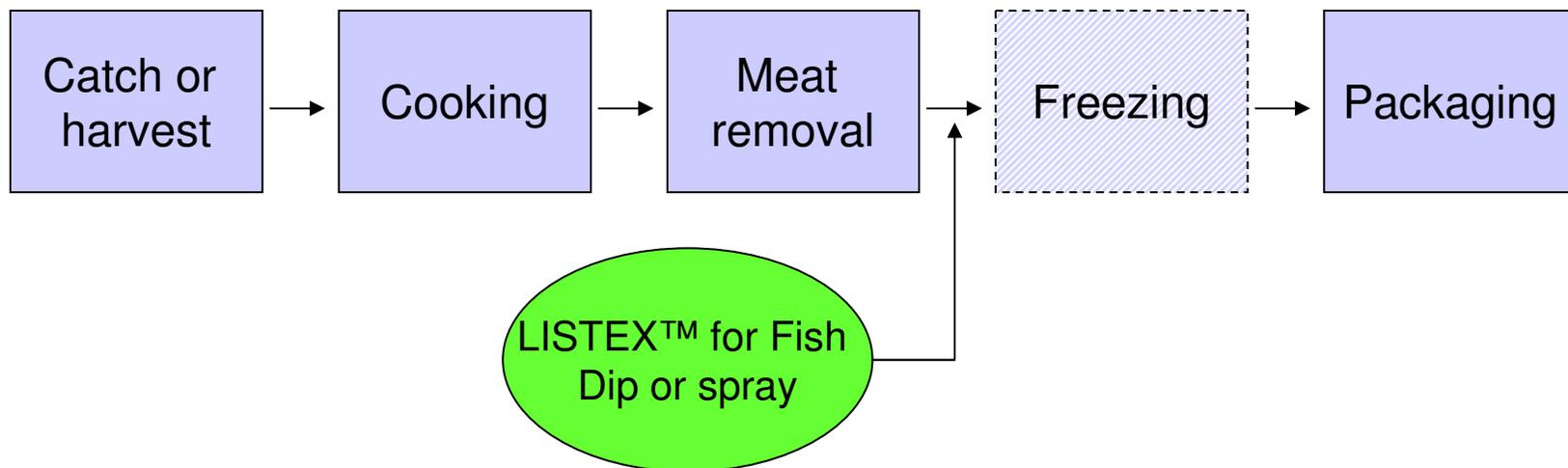
Calculation :  $1900 \times 5 \times 10^7$  (=appr.  $1 \times 10^{11}$  pfu/fillet  
of 2kg; 1 bottle for 400 kg = 200 fillets)

\*pfu: plaque forming unit



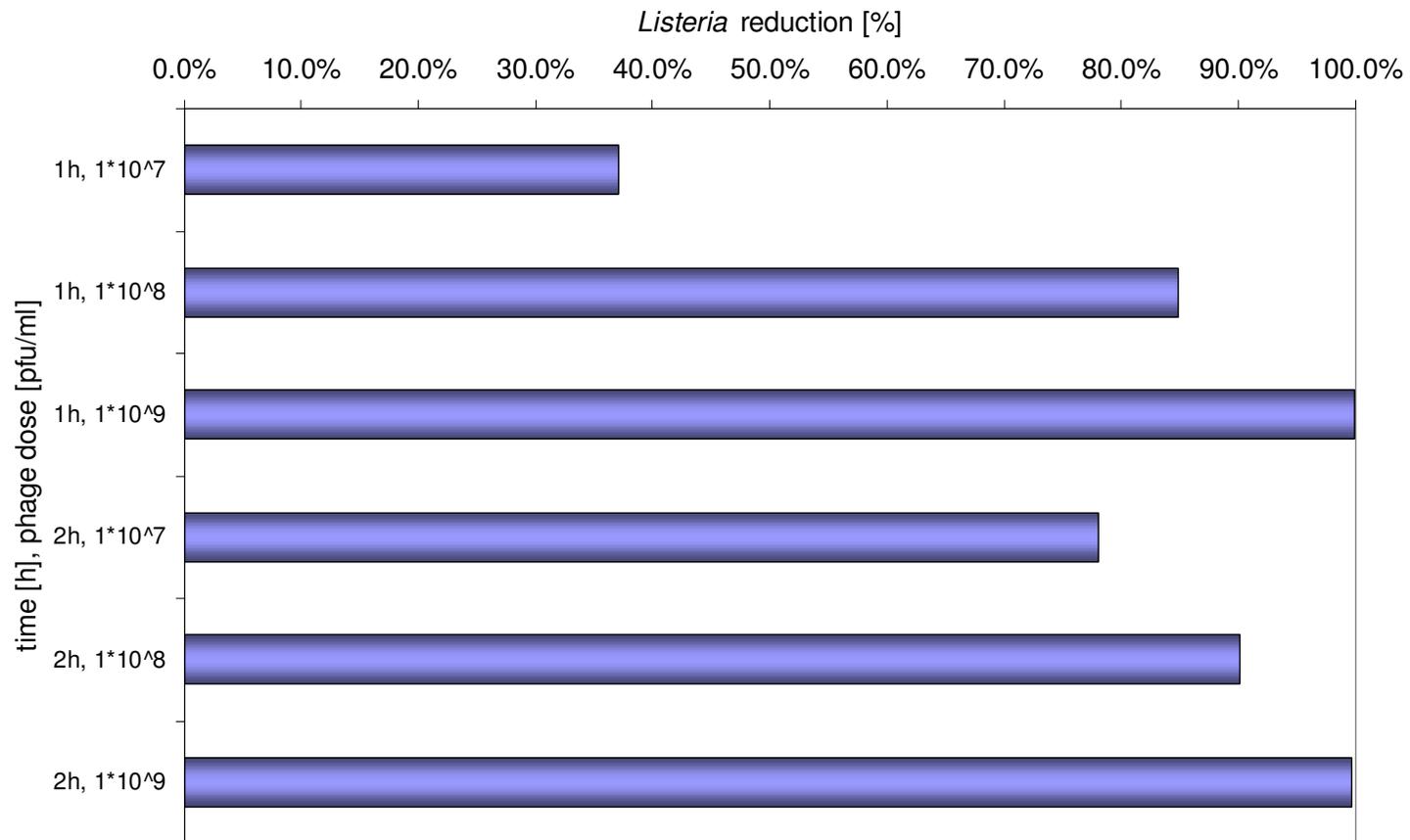
## Seafood Application

- Seafood can be treated with LISTEX after intensive (often manual) handling: the meat removal.
- LISTEX avoids cross contamination and removes Listeria from the meat.



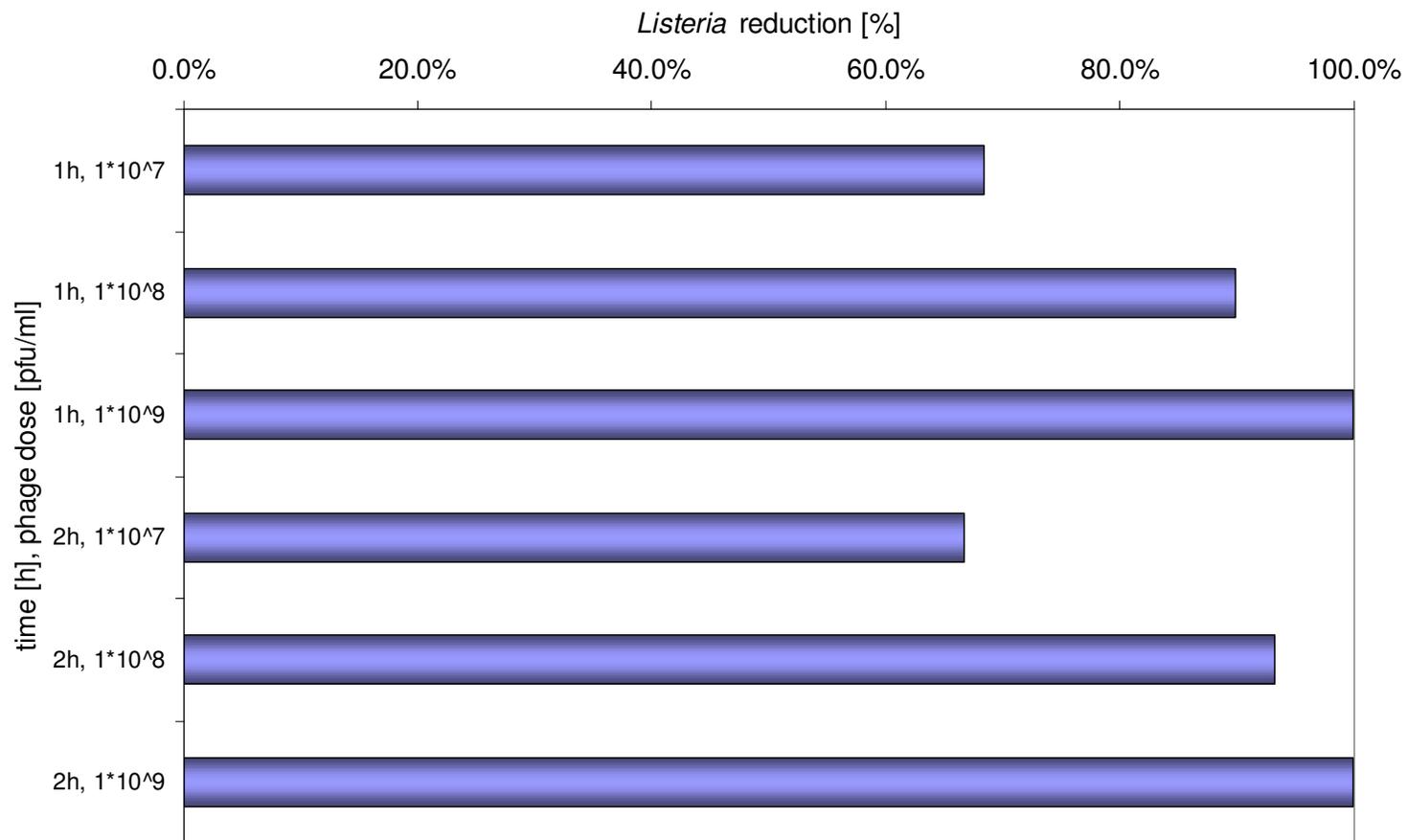


# Up to 100% Listeria reduction on cooked peeled shrimp



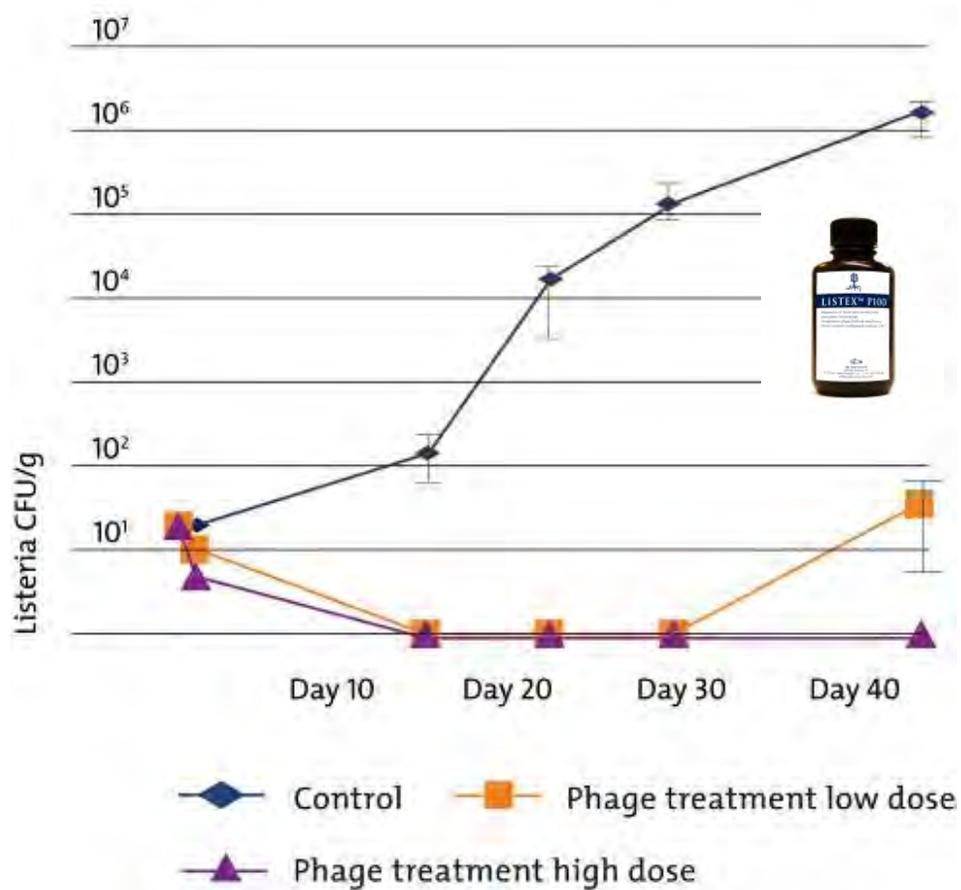


# Up to 100% *Listeria* reduction on cooked mussels



# Meat

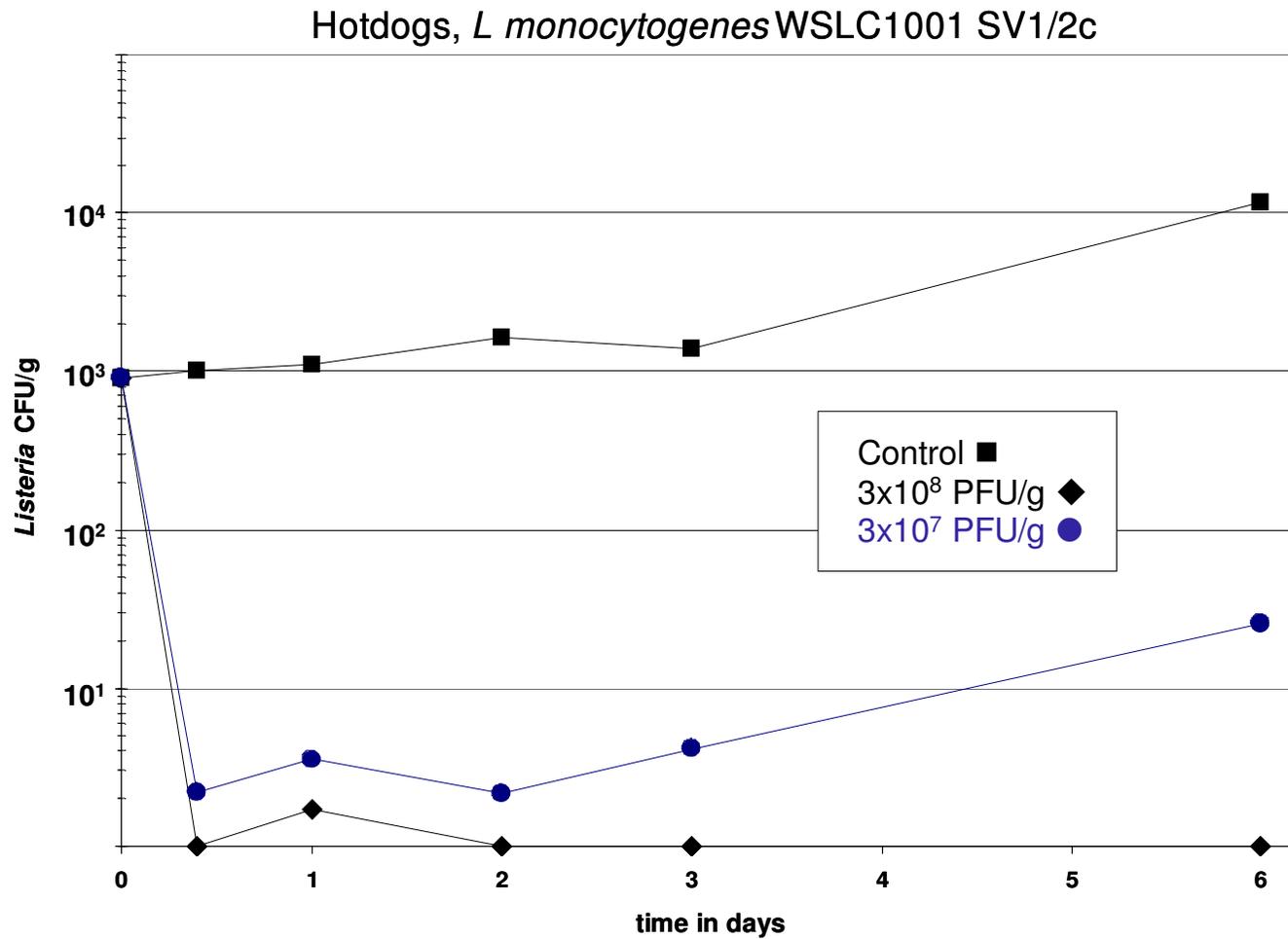
University of GENT: Dose dependant control of *Listeria* on cooked meat product ( $1 \times 10^7$  and  $5 \times 10^7$  pfu/cm<sup>2</sup>)





# Hot dogs

Data generated by ETH (Swiss Federal Institute of Technology Zurich)





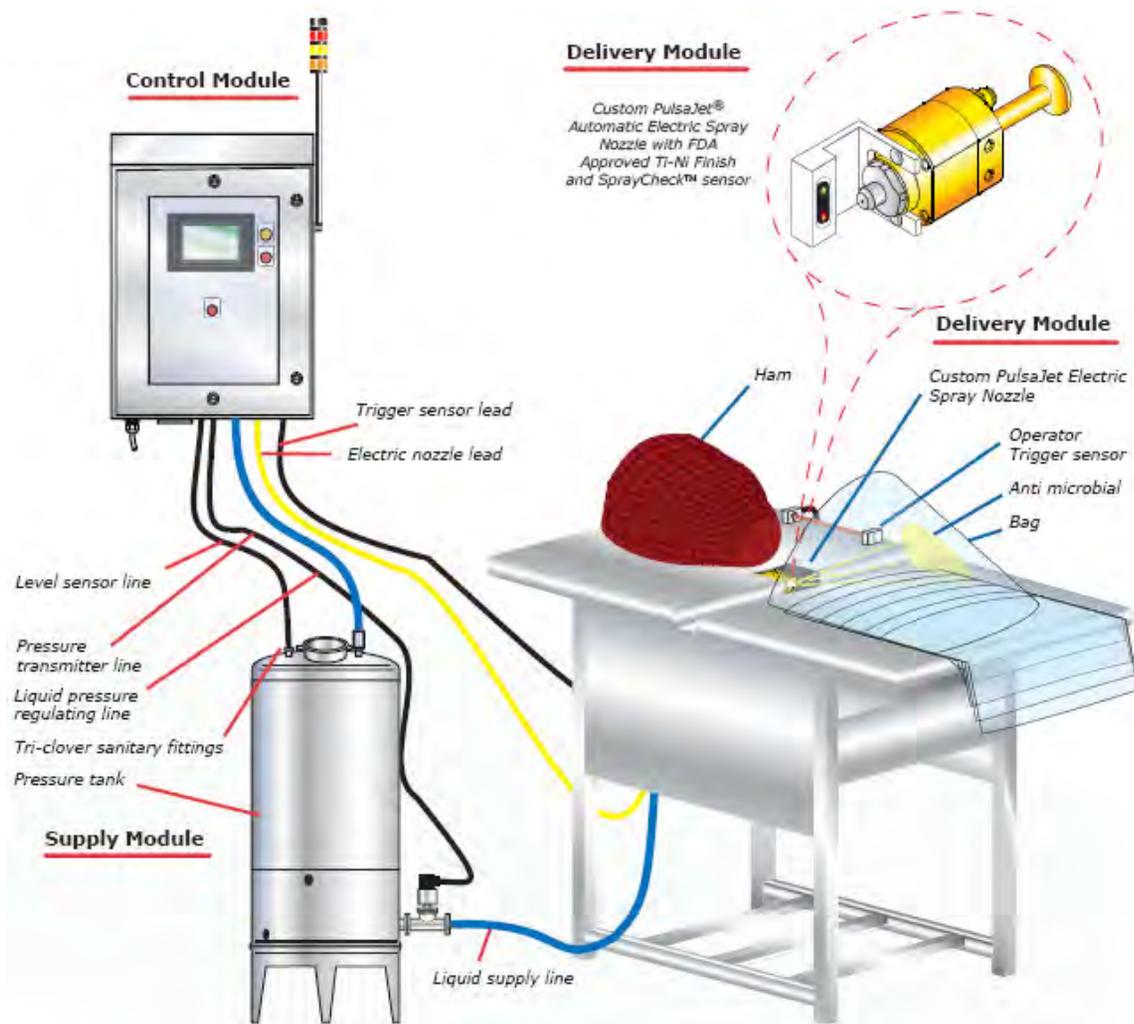
## Meat – Application example

Treatment of whole hams:

- Hams: 45 cm x 35 cm x 10 cm/4,5 kg weight
- Shoulders: 30 cm x 30 cm x 8 cm/2,5 kg weight
- Target dosage:  $2 \times 10^7$  pfu/cm<sup>2</sup>
- Amount of water that can be deposited on this surface: 1 ml/400 cm<sup>2</sup>
- Advised working solution:  $8 \times 10^9$  pfu/ml
- 4% dilution, add 40 ml of LISTEX™ per liter water



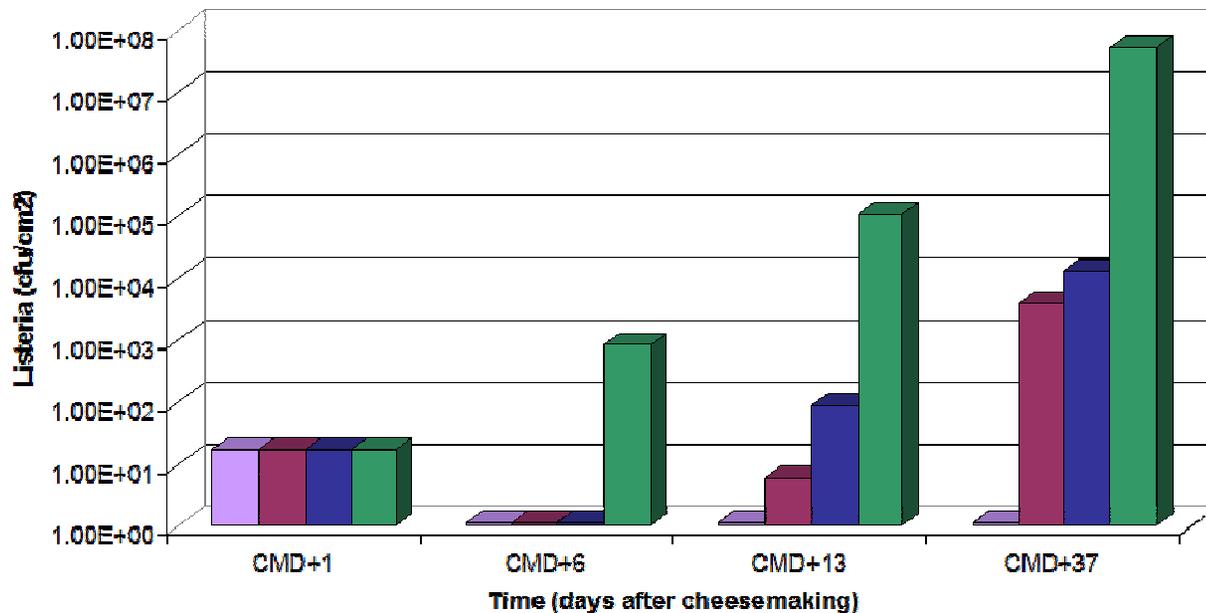
# Spraying – Equipment example





# Cheese

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



Initial contamination:  
~20 cfu/cm<sup>2</sup>

Treatments:

$6 \times 10^7$  pfu/cm<sup>2</sup>

$1 \times 10^7$  pfu/cm<sup>2</sup>

$3.5 \times 10^6$  pfu/cm<sup>2</sup>

Control

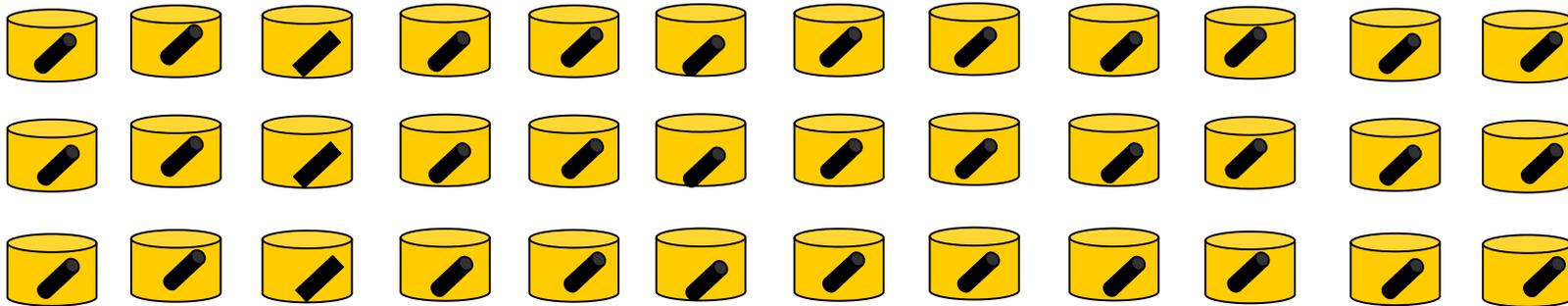


# Cheese

- Ripening period is the problem
- Cross-contamination
- Low incidence - high numbers in the final product
- Mostly surface ripened cheeses



## Cross contamination by the smear solution



- Smear cheeses especially vulnerable
- Initial “preemptive curative” treatment(s)
- Protect smear solution



## Cheese – Application example

- Cheeses (gorgonzola): D: 33 cm, H: 25 cm
- Cheeses can be sprayed or dipped
- One or two doses, second dose after a few days
- Treatment:  $4 \times 10^7$  pfu/cm<sup>2</sup>
- Calculate surface = 4302 cm<sup>2</sup>
- Calculation:  $4302 \text{ cm}^2 \times 4 \times 10^7 \text{ pfu/cm}^2 = 1.7 \times 10^{11}$  pfu  
(approximately 1 ml LISTEX™ per cheese)



## Liquids – Application example

- Feta cheese in brine (cheese stays in brine for a number of days)
- Brine is 4 liters (4000 ml)
- Advised dosage is  $1 \times 10^8$  pfu/ml
- Calculation:  
 $4000 \text{ ml} \times 1 \times 10^8 \text{ pfu/ml} = 4 \times 10^{11} \text{ pfu} = 2 \text{ ml}$   
LISTEX™ in 4 liter brine.



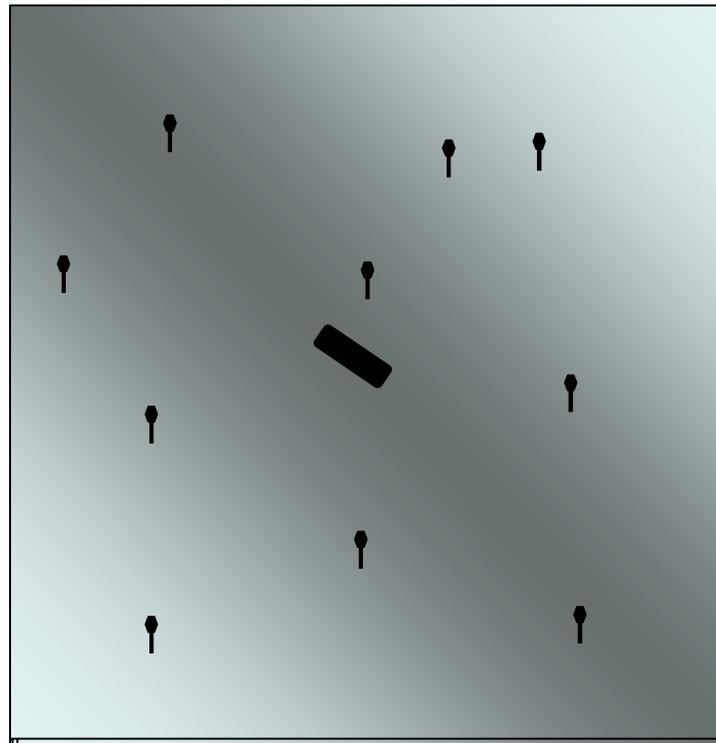
## How to determine the dosage?

- Universal dosage LISTEX™ =  $2 \times 10^8$  pfu/cm<sup>2</sup>
- Phages move in water by diffusion (quicker in wet applications)
- Phages need time to find the *Listeria*
- Did the *Listeria* get time to adapt?
- Contamination level / reduction goal
- High surface to weight ratio needs more phages per kilogram
- Above factors will determine the right dosage; for example  $5 \times 10^7$  pfu/cm<sup>2</sup>



A closer look at the dose on 1 millionth of a square cm

•  $10^3$  pfu/cm<sup>2</sup>





## Things to know about LISTEX™

- $2 \times 10^{13}$  phages per bottle of 100 ml
- 10 bottles/box, delivery within two weeks
- Store refrigerated; shelf life 6 months
- SKAL (Dutch organic production inspection authority) organic processing aid approved
- Processing aid
- Kosher
- Allergen free





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**Thank you!**

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