

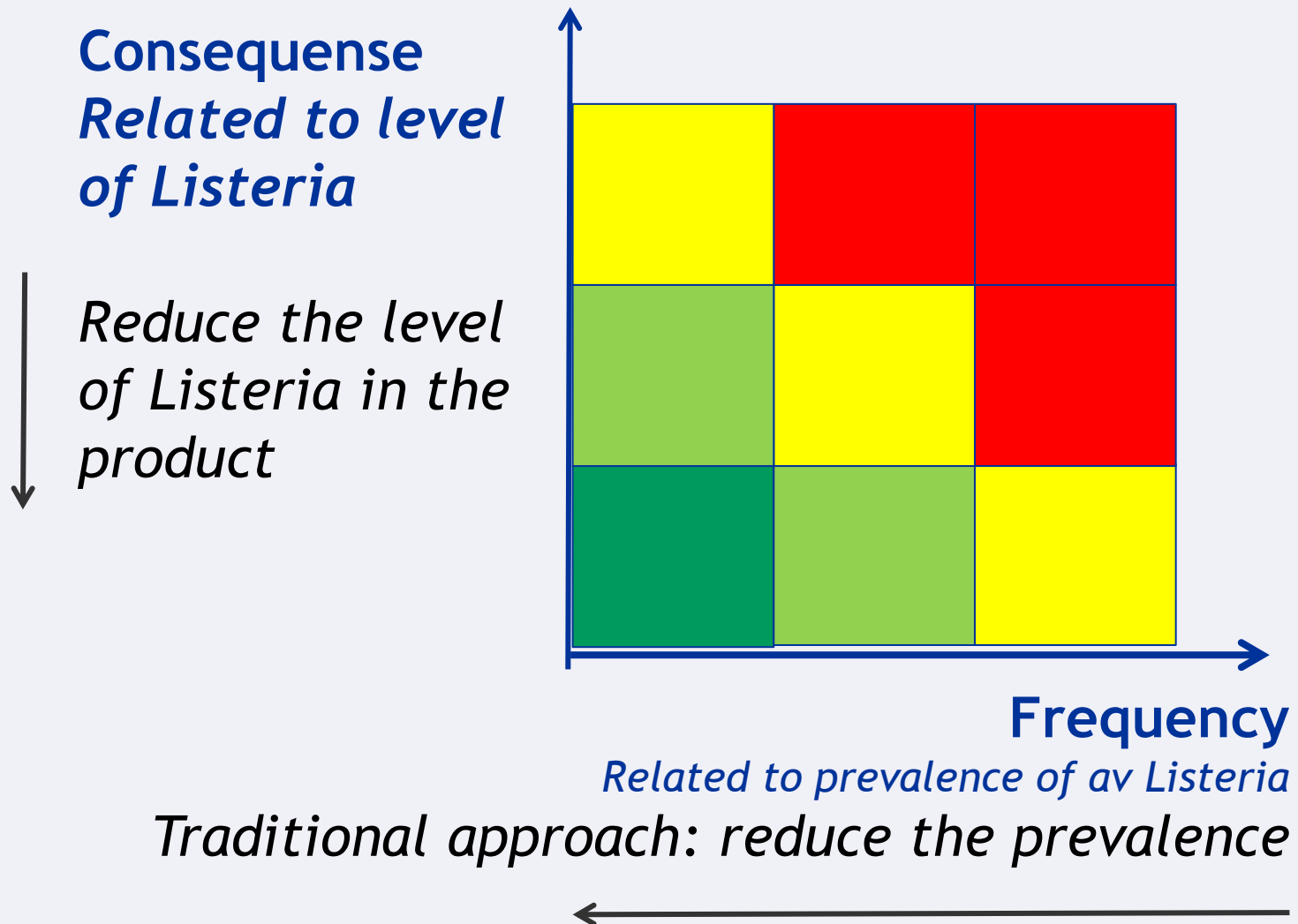
Hvor mye *Listeria* kan det være i norsk laks?

Taran Skjerdal, Veterinærinstituttet

Hvor mye Listeria kan det være i laks...

- ..Uten at det går utover mattryggheten?
- ..rett etter filetering
- ..for at den skal holde kravene i regelverket på siste forbruksdag
- ..for at den skal kunne brukes til sushi og sashimi
- ..for at man skal kunne påvise den med vanlige analysemetoder
- ..for at den skal kunne brukes til røykelaks

Risk = frequency x consequence:
New approach: Consequence more focused



Food category	Micro-organisms/their toxins, metabolites	Sampling-plan ⁽¹⁾		Limits ⁽²⁾		Analytical reference method ⁽³⁾	Stage where the criterion applies
		n	c	m	M		
1.1. Ready-to-eat foods intended for infants and ready-to-eat foods for special medical purposes ⁽⁴⁾	<i>Listeria monocytogenes</i>	10	0	Absence in 25 g		EN/ISO 11290-1	Products placed on the market during their shelf-life
1.2. Ready-to-eat foods able to support the growth of <i>L. monocytogenes</i> , other than those intended for infants and for special medical purposes	<i>Listeria monocytogenes</i>	5	0	100 cfu/g ⁽⁵⁾		EN/ISO 11290-2 ⁽⁶⁾	Products placed on the market during their shelf-life
		5	0	Absence in 25 g ⁽⁷⁾		EN/ISO 11290-1	Before the food has left the immediate control of the food business operator, who has produced it
1.3. Ready-to-eat foods unable to support the growth of <i>L. monocytogenes</i> , other than those intended for infants and for special medical purposes ⁽⁴⁾ ⁽⁸⁾	<i>Listeria monocytogenes</i>	5	0	100 cfu/g		EN/ISO 11290-2 ⁽⁶⁾	Products placed on the market during their shelf-life

⁽¹⁾ n = number of units comprising the sample; c = number of sample units giving values over m or between m and M.

⁽²⁾ For points 1.1-1.24 m=M.

⁽³⁾ The most recent edition of the standard shall be used.

- **Max 100 cfu/g på siste forbruksdag**
- **Skiller mellom produkter der *Listeria* kan vokse og ikke kan vokse**
- **Kriteriene gitt ut fra produktprøver**
- **Er produktprøver nyttige – til hva?**
- **Trendanalyser-beslutningsgrunnlag – tilbaketrekking?**



salmon, seabass, tuna etc



One PO for chilled fish, independent of "application"?

Traditional products

gutted fish,
fillets,
cotelettes

heavily
processed
products
- surimi

raw products,
short shelf life

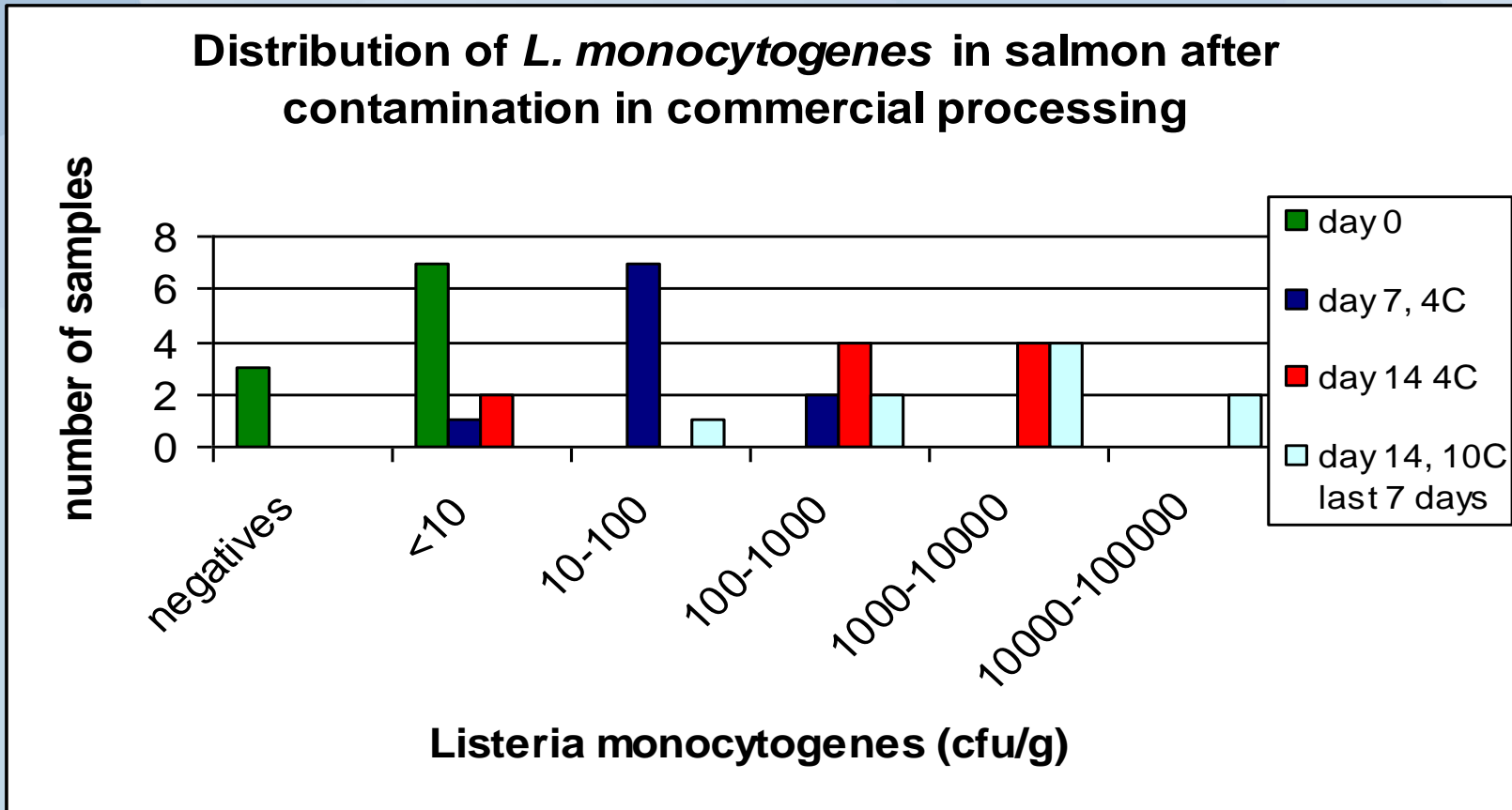
- sushi
- carpaccio

raw products,
long shelf life

-smoked fish
-double
frozen fish



Results from 1 batch of naturally contaminated fresh salmon, 10 X 4 samples

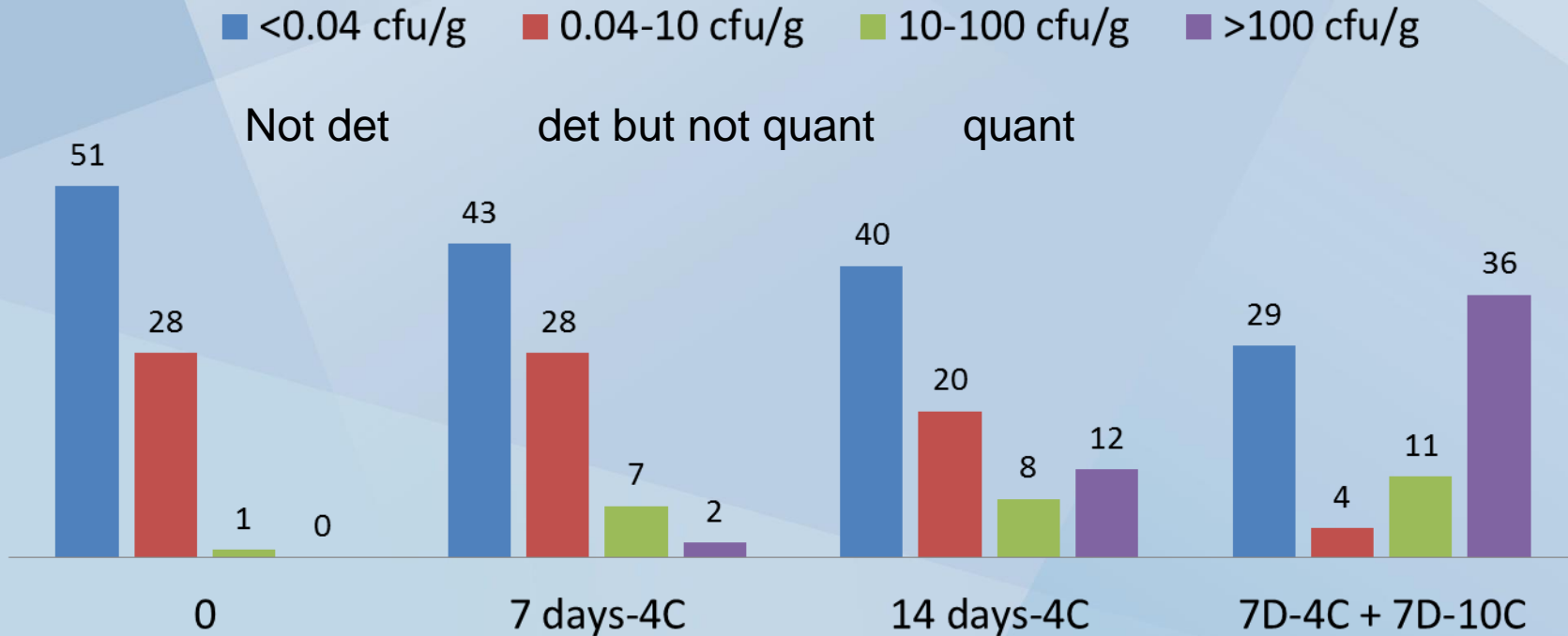


***Listeria* grows in naturally contaminated salmon at 4°C!**

Extreme variation - high levels during storage even

if no samples had more than 10 cfu/g at day 0

Repeated analyses with 8 batches, 320 samples, measured levels of L.m



- **Many samples above 100 cfu/g at day 14, even though the levels were below/at detection level at day 0**
- **Doubling time at 4C: app 1.6 days.** More rapid growth in fresh salmon than in cold smoked salmon.

PO for day 0 is needed, but what is the real level at day 0?

Growth kinetics – back to basic

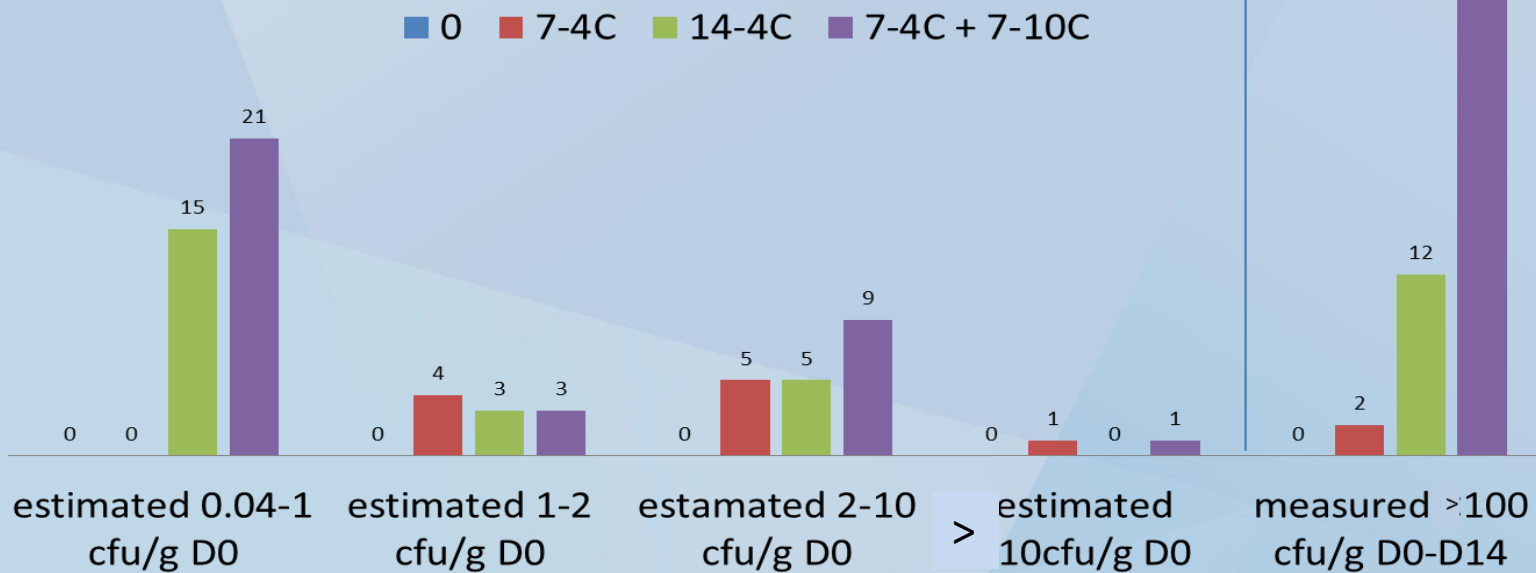


doubling times	cfu/g	
0	100	FSO-limit value on the last day of shelf life
1	50	
2	25	
3	12,5	
4	6,25	10 cfu/g detection level quantitative analysis method
5	3,13	
6	1,56	
7	0,78	1 cfu/g
8	0,39	
9	0,20	
10	0,10	
11	0,049	1 cfu/25 g detection level qualitative analysis
12	0,024	
13	0,012	
14	0,006	1 cfu/150 g
15	0,003	
16	0,002	1 cfu/kg
17	0,001	

The final L.m level depend on initial level, storage time and temperature. Levels <10 cfu/g are not 0!

Estimated *L. monocytogenes* levels in naturally contaminated salmon, day 0 (traced back)

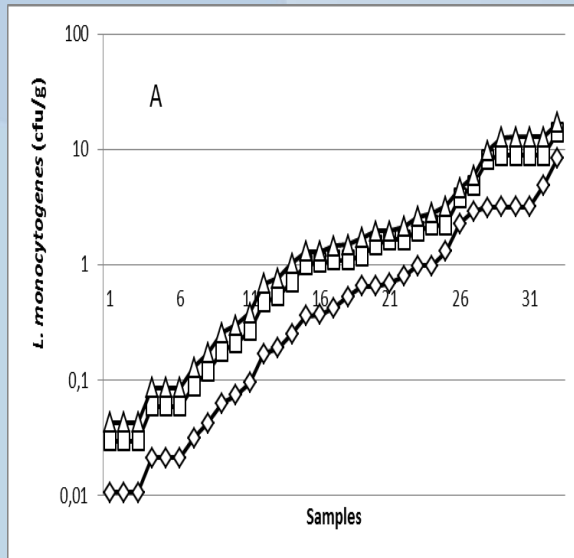
based on growth rate and data from day 7-14 (td at 4 C is 1.6 days, at 10C app 1 day)



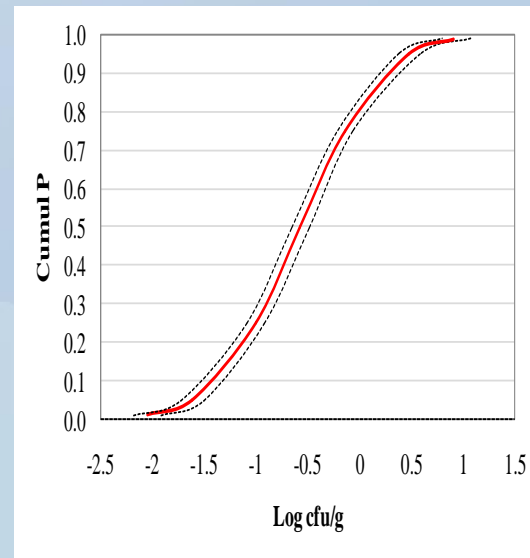
- **Most samples seemed to contain very low levels on day 0**
- **Median value of positive samples: app 1 cfu/g**

Estimation of *Listeria* levels in naturally contaminated salmon at the processing day, 3 approaches

1. **Extrapolation** based on measured growth rates from experiments and models



2. Estimated distribution at day 0, **statistical methods** (WP6)



3. **Analyses of 15 new samples**, detection levels 2 cfu/g

- 1 sample: 2 cfu/g
- 12 samples: between 1 cfu/25 g and 2 cfu/g
- 3 samples below 1 cfu/25 g

Systematic variations

- Morning samples different from Late day samples
- Winter ulcer season: similar (or lower) prevalence and levels of *Listeria*
- Local contamination remains to a large extent local. If *Listeria* spreads: mainly downwards.

Suggested PO values for fresh salmon (and sea bass), day 0 based on intended use and realistic storage scenario:

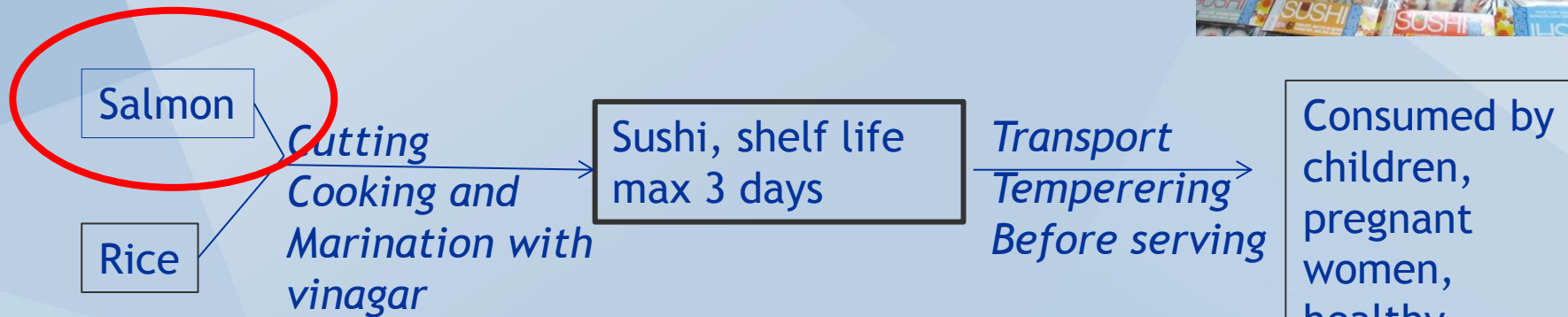
Criterion 1: max 100 cfu/g on the last day of shelf life.

Intended use of salmon	Ice storage or frozen	4°C for 7 days	4°C for 14 days	4°C with periods at abuse temperature
Raw	10-50 cfu/g	5-8 cfu/g	<2 cfu/g	Absence in 25 g

Hvor mye Listeria kan det være i laks etter filetering uten at det går ut over mattryggheten:

Det kommer an på hva den skal brukes til, og hvordan den lagres fram til bruk

Sushi prepacked in tray

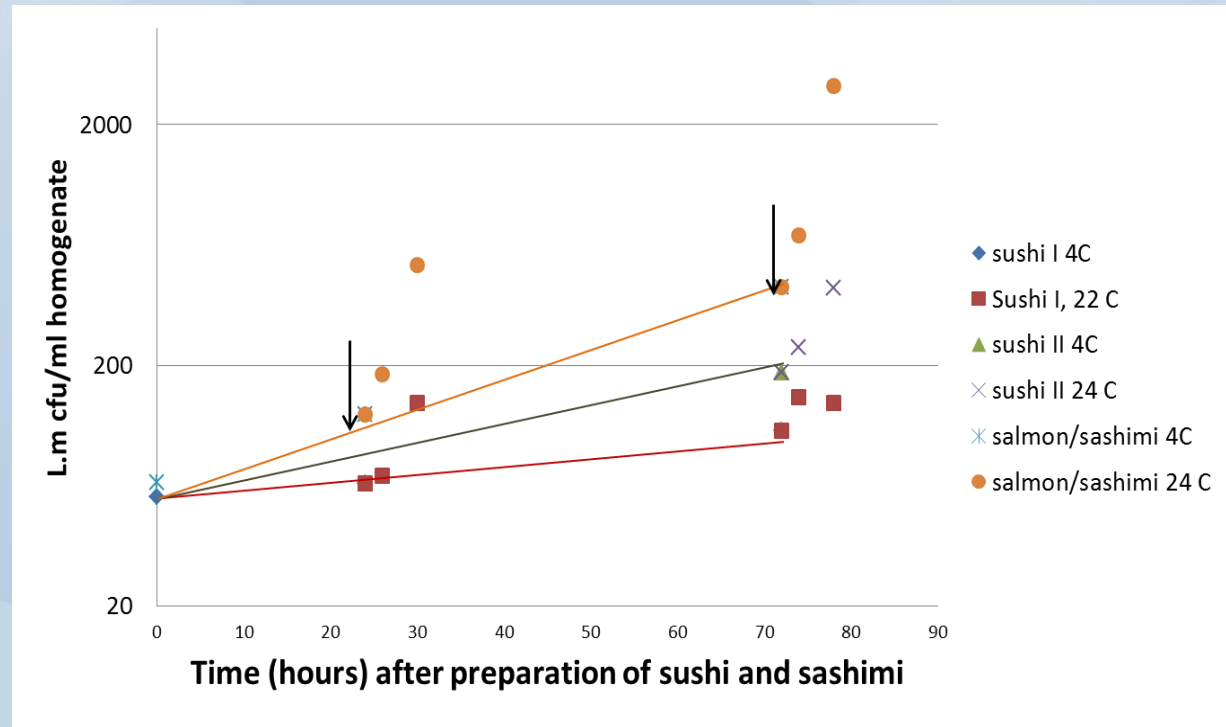


- *Salmon traditionally cooked before consumption.*
- *Sushi has a short shelf life, shelf life studies for risk assessment of Listeria not needed, but...*
- *What if the raw salmon has Listeria,*
 - *will sushi made from it be safe to eat?*
 - *How much Listeria can be tolerated in salmon before the food safety of sushi is compromised?*

Listeria monocytogenes in sushi

Growth of *L.m* in sushi and salmon/sashimi

Significant growth in sashimi (raw fish in slices), but limited growth in sushi, probably due to acidic conditions.



POs for fish intended used for sushi and sashimi

- will depend on freshness of the raw material and recommended procedure for temperature adaptation.
- can be developed using the same approach as for fresh salmon,

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Criterion 1: max 100 cfu/g on the last day of shelf life.

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Raw	10-50 cfu/g	5-8 cfu/g	<2 cfu/g	Absence in 25 g
Sushi, including tempering period	≤10 cfu/g	1-2 cfu/g	Absence in 25 g	Absence in 25 g
Sashimi, including tempering period	≤5 cfu/g	0.5-1 cfu/g	Absence in 25 g	Absence in 25 g

Do salmon need to be Listeria free to make sushi safe?

No, but the Listeria levels must be «far below» 10 cfu/g, in the entire batch.

Sampling procedures and analysis methods needs to be adapted

...what about smoked salmon

Protocol to obtain 2 cfu/g detection level

Sample preparation:

1. 1 part product + 1 part diluent (BPV or Half Fraser Broth)
2. Recusitation period, as in the ISO method
3. Spread 1 ml on 3 ALOA (or 2 ml on 6 plates), as normal

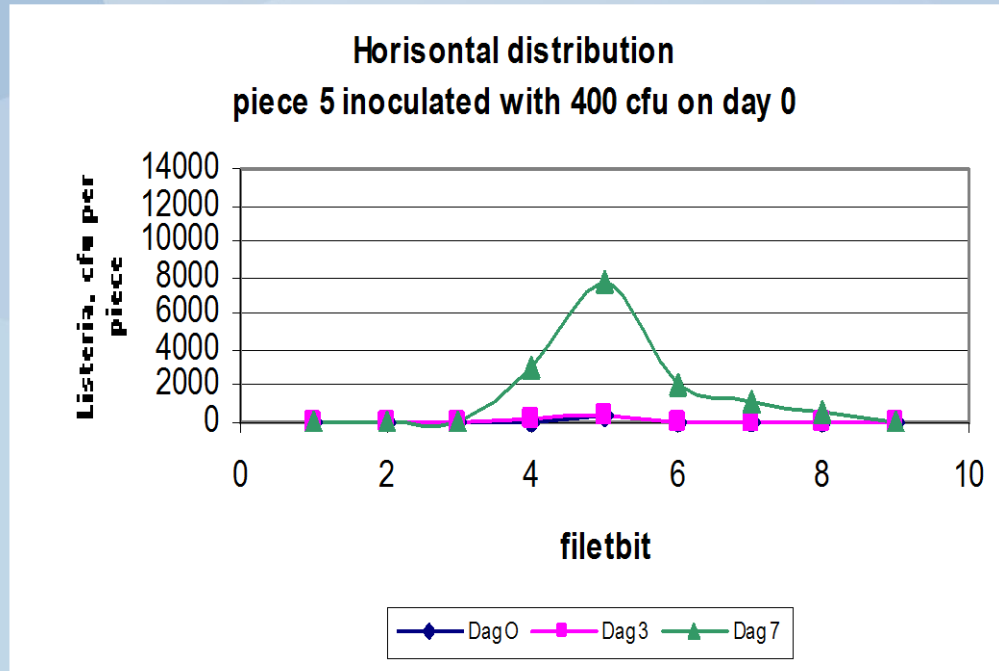
Food sampling - not sufficient to ensure food safety if applied alone

Probability not to detect *Listeria* in a lot if only a part of it is contaminated

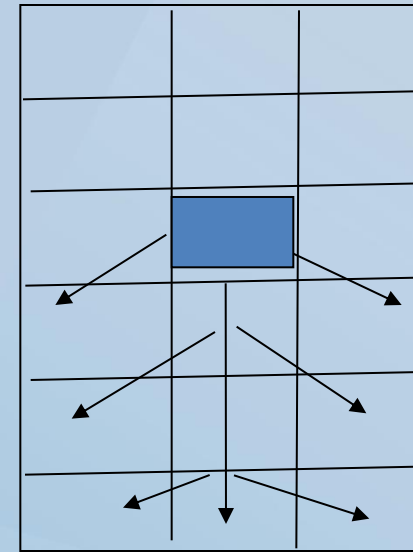
Number of sample units tested	1 % contaminated with <i>Listeria</i>	5 % contaminated with <i>Listeria</i>	10 % contaminated with <i>Listeria</i>
5	0,95	0,77	0,58
10	0,90	0,60	0,35
15	0,86	0,46	0,21
20	0,82	0,36	0,12
30	0,74	0,21	0,04
40	0,67	0,13	0,01
50	0,61	0,08	0,01



Model experiments to investigate distribution of Listeria from 1 inoculated piece



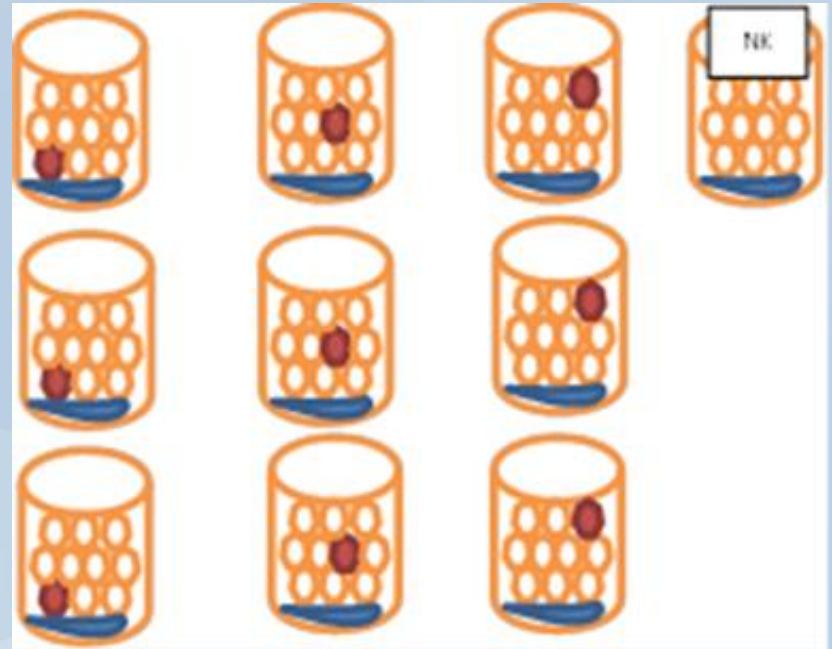
Vertical distribution



Sampling on the bottom water fraction tested with naturally contaminated salmon in one experiment:
L.m detected after 7 and 14 days of storage even when when all fishes (25 grams samples) were negative.

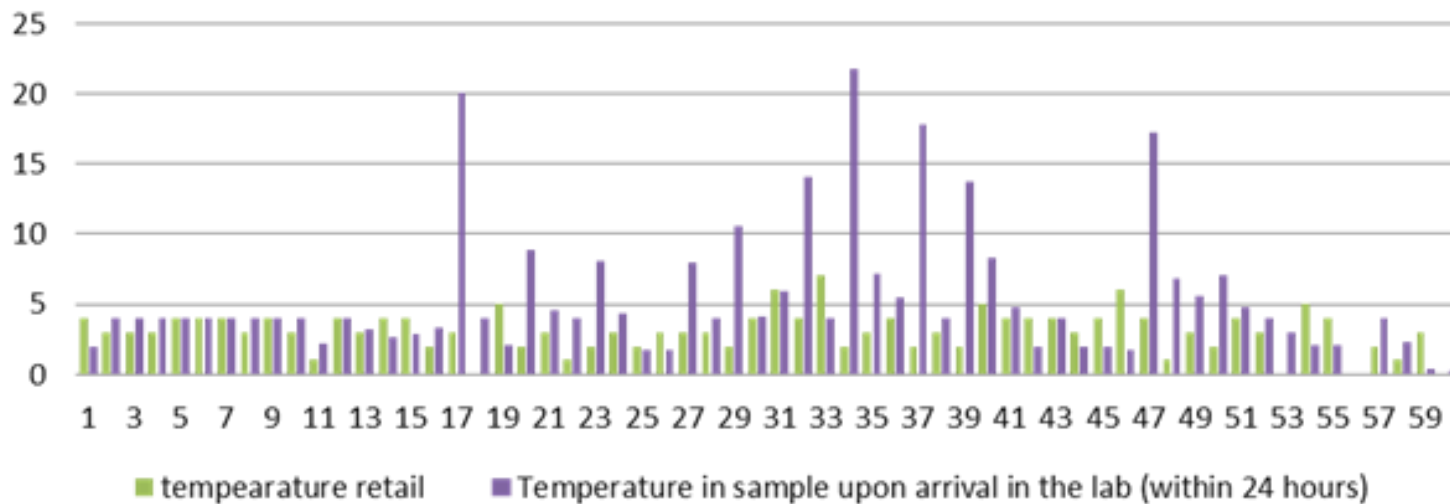
Sampling for low and unevenly distributed Listeria

- Suitable sampling points for detection: bottom layers of fish or washing solution of pooled samples
- *We have obtained a prototype sampling method and analyse method with detection level 1 cfu/5 grams*



Temperature during transport of sample to the lab

Temperatures at retail and transport of samples, January-December, 5 samples per month



- *Abuse temperature during transport of samples occurs!*
- *Introduces a risk of overestimation of the Listeria level*

Hvor mye Listeria kan det være i laks...

- ..Uten at det går utover mattryggheten?

Det kommer an på hva den skal brukes til, og hvordan fisken lagres

- ..rett etter filletering

I anlegg med Listeria, men svært god hygiene: vanligvis godt under 10 cfu/g. I vårt testtilfelle: median av positive prøver 1 cfu/g

- ..for at den skal kunne brukes til sushi og sashimi
Ikke mer enn 2 cfu/g for sushi, lavere for sashimi.

Hvor mye Listeria kan det være i laks...

- ..for at den skal holde kravene i regelverket på siste forbruksdag

Avhenger av tid og temperatur, se tabell

- ..for at man skal kunne påvise den med vanlige analysemetoder

*1/25 gram i kvalitativ test, 1/10 g i kvantitativ test.
Mer sensitive metoder er på gang.*

- ..for at den skal kunne brukes til røykelaks

Listeria vokser svært langsomt i røykelaks, tåler større startkonsentrasjon av Listeria enn for sushi.

Thanks for invitation and attention!

For more information, see <http://www.baselineeurope.eu/>

TOPPTEKST

Hvor mye Listeria kan det være i fersk laks for at den skal kunne spises rå?

Det var mange som ønsket å lære mer om trykghetskrav og prøvetaking for Listeria i laks da resultatene fra EU-prosjektet Baseline ble presentert på et seminar ved Veterinærinstituttet i juni. Hvorfor er Listeria et så stort problem i næringsmiddelindustrien, og hvordan kan man gå fram for å håndtere risikoen dersom Listeria finnes i produksjonsmiljøet og i ferdig produkt?

Av Elin Reitehaug og Taran Skjerdal, Veterinærinstituttet

En stadig større del av befolkningen både i Norge og verden for øvrig konsumerer laks i rå tilstand fremfor å varmebehandle den før konsum, og man kan stille seg spørsmålet: skal laks anses som spiseklar mat? Skal man i så tilfelle stille mål for hal laks, filetstøt, filetfister eller ferdige

over de store tilf. Listeria finnes nær sagt overalt – i bakterien finnes naturlig i vann, jord, planter og dyr. Listeria i mat kan komme både fra råvarer, fra matvarer ved kontakt med eller fra produksjonsmiljøet. Listeria kan vokse ved kjøletemperatur, både med og uten tilgang på oksygen, og den tåler høye konsentrasjoner av salt. Dessuten kan Listeria etablere seg i pro-

dukt i Europa (ANSES) har ledet arbeidet, og Veterinærinstituttet har deltatt i arbeidsgruppen. Retningslinjene har blitt prøvd for laks og sushi i Baseline-prosjektet. Det vil si at resultatene som presentasjonen kan brukes som dokumentasjon på at det gjøres risikovurdering av disse produktene.

Retningslinjer og kriterier for Listeria

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Development of performance objectives for *Listeria monocytogenes* contaminated salmon (*Salmo salar*) intended used as sushi and sashimi based on analyses of naturally contaminated samples

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