Impact of pooling pre-enriched test portions on the detection of *L. monocytogenes* in food.

9th workshop of the NRLs for *L. monocytogenes*
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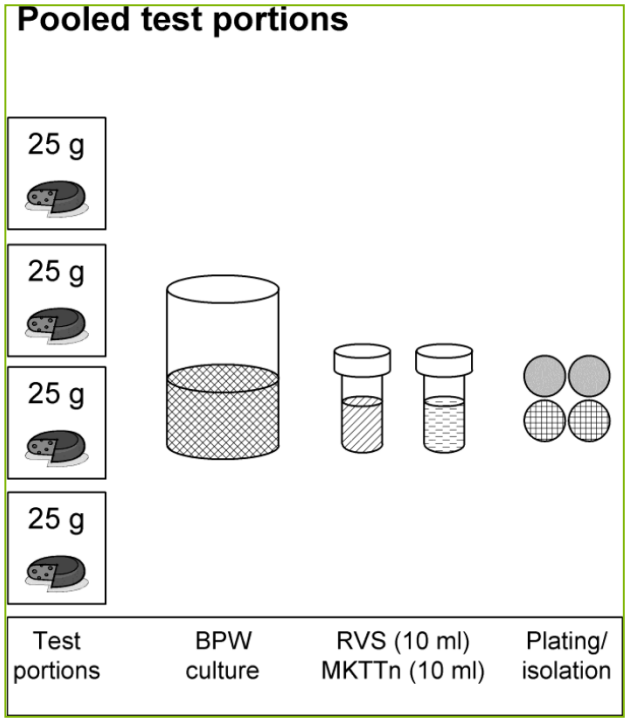
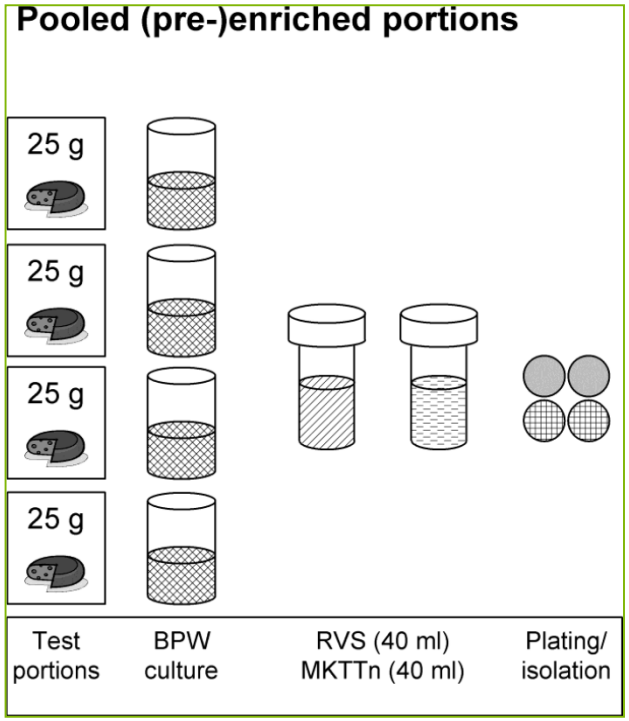
ISO/DIS 6887-1
Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - General rules

- **Clause 9.3: Pooling and compositing procedures for qualitative tests, Annex A**





- **Annex D: Verification protocol**

Little reliance should be placed on the results of a single trial and the chosen protocol should be repeated at least 5, and ideally 8 to 10, times using different samples of the same matrix type/target microorganism combination.

Impact of wet pooling samples on the performance of EN ISO 11290-1 Standard

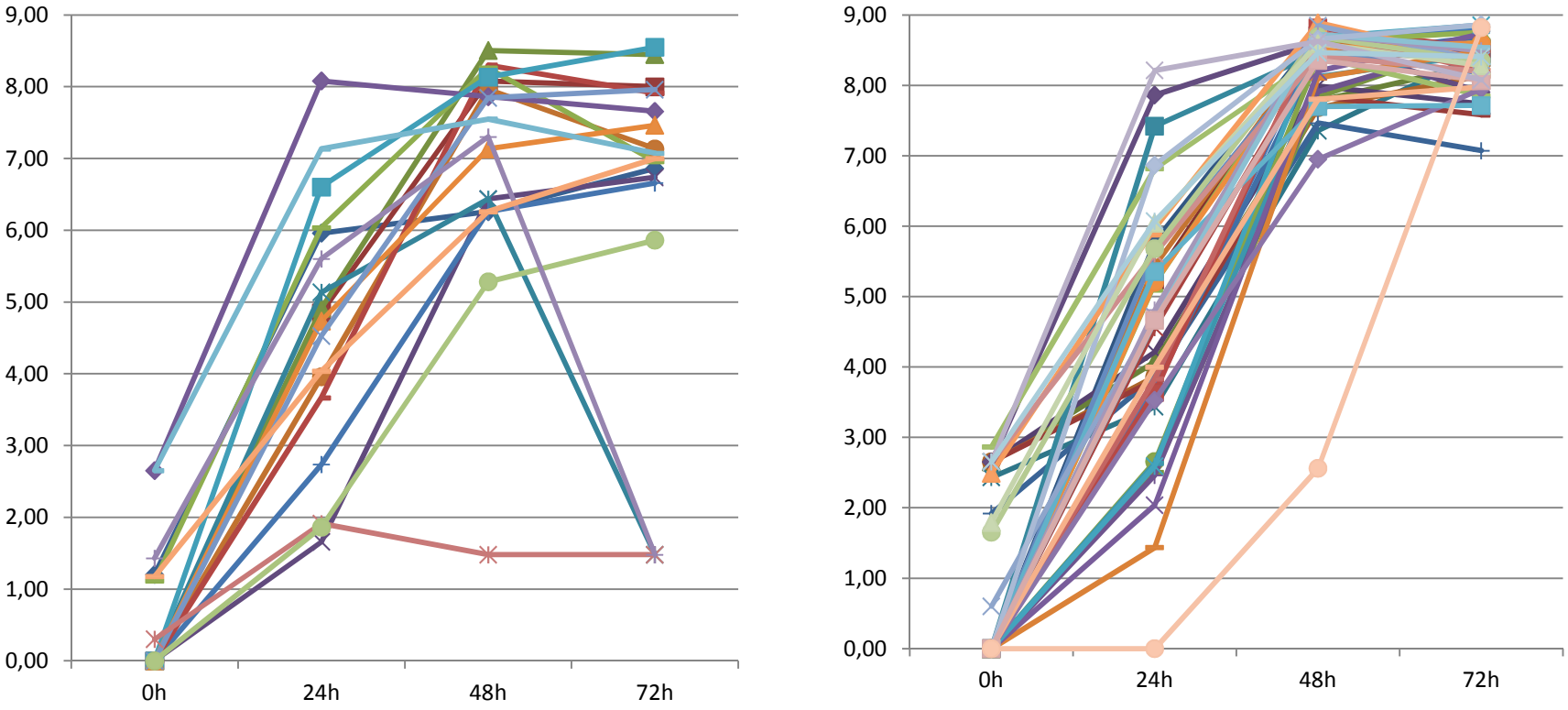
- Development and validation of a modelisation of *L. monocytogenes* growth along pre-enrichment in half-Fraser

- Use of the model to estimate loss of sensitivity in case of pooling

Evolution of *L. monocytogenes* populations in naturally contaminated food samples undergoing enrichment culturing and possibility to reduce 2nd enrichment duration

(Gnanou Besse et al. 2015 submitted)

- **Enumeration of enrichment broths of 77 naturally contaminated samples**



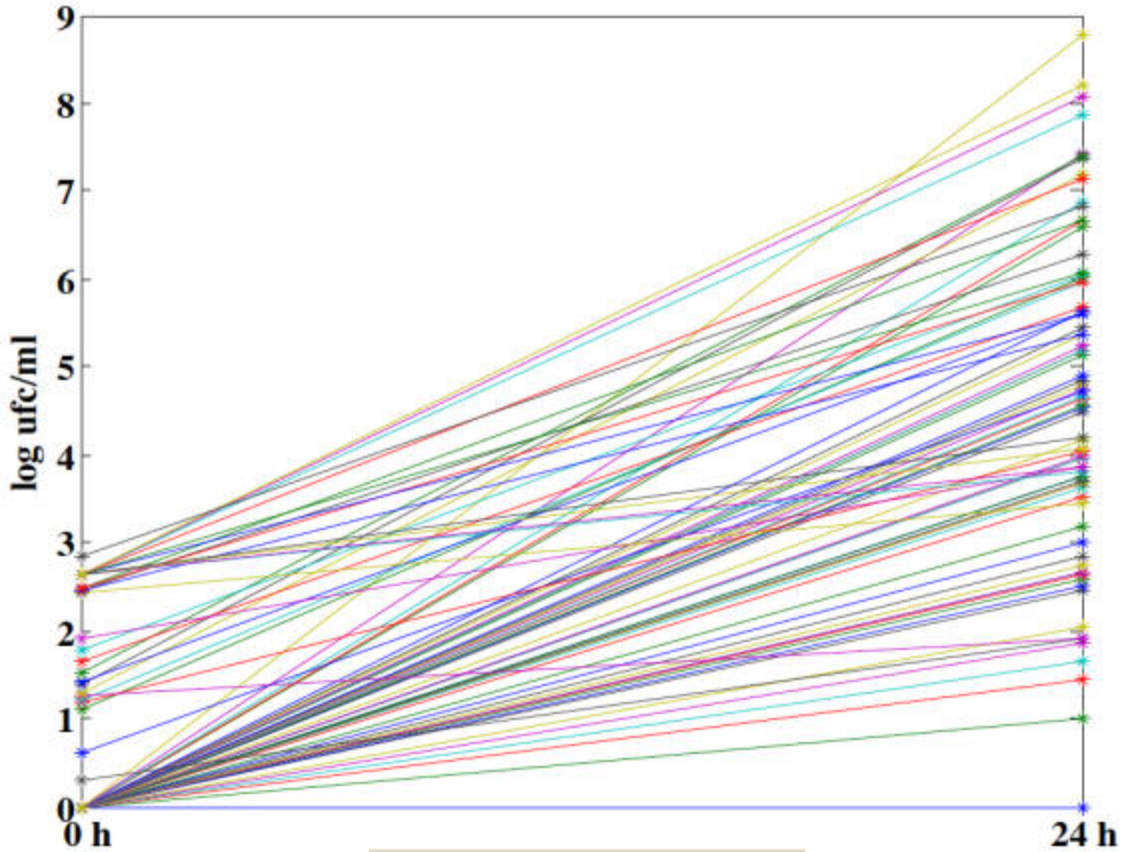
Evolution of *L. monocytogenes* population

With other *Listeria* species

Without other *Listeria* species

Half-fraser

- Observed increase

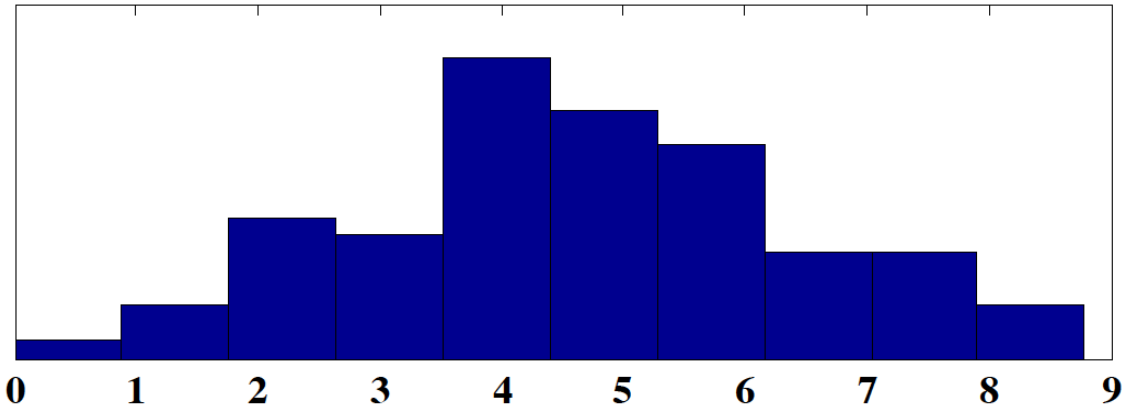


Half Fraser enrichment

- Concentration at 24 h = [0-8.8] log cfu/ml

Half-fraser

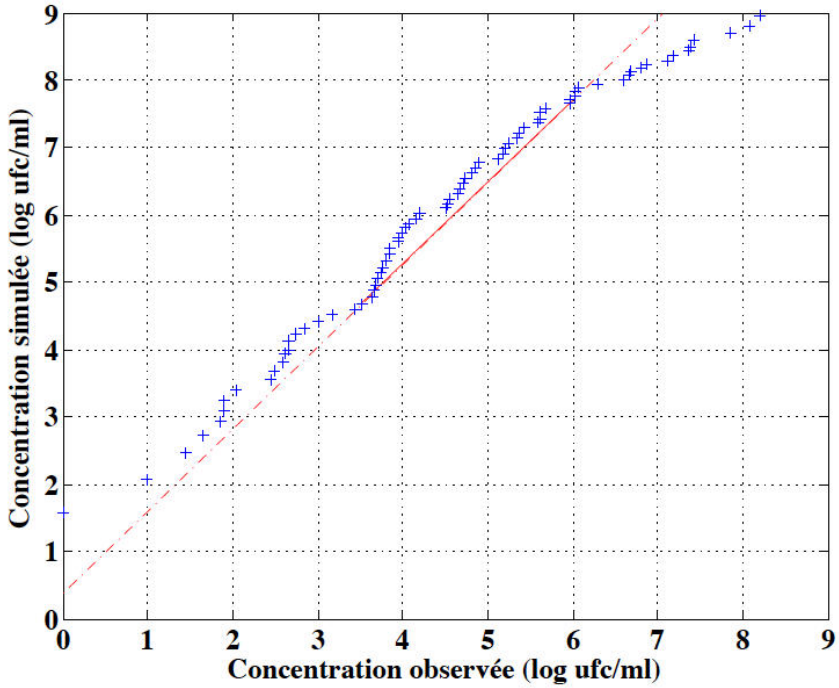
- Concentration at 24h (log $_{10}$ cfu/ml)



- Variability depends on :
  - Initial concentration (Ci)
  - Growth rate
  - Initial physiological stage (probability to multiply and lag)
  - N max

Modelisation of growth

- Variability of Ci, growth rate (34 values) and Nmax

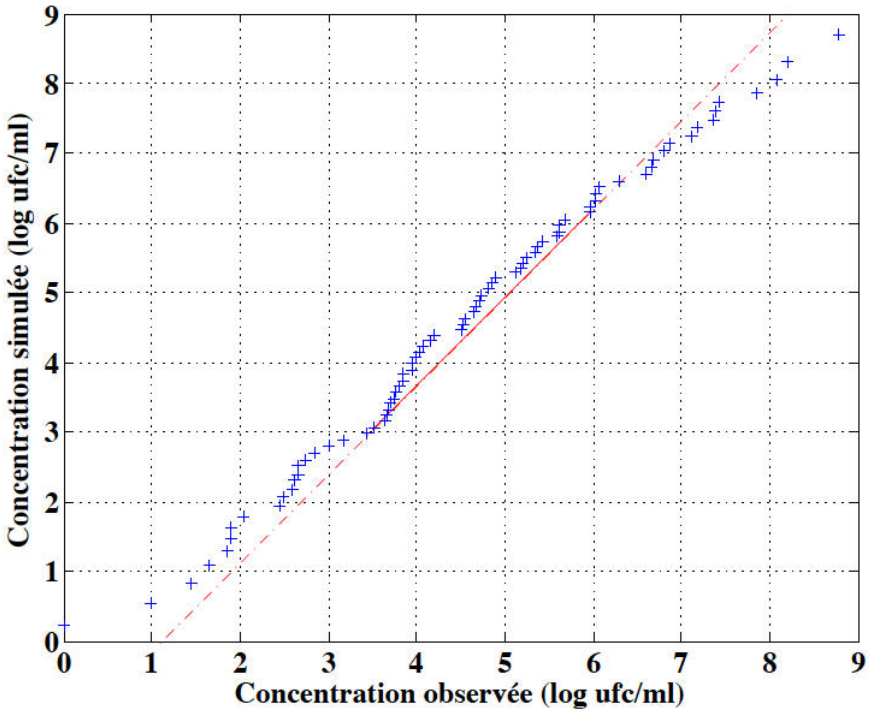


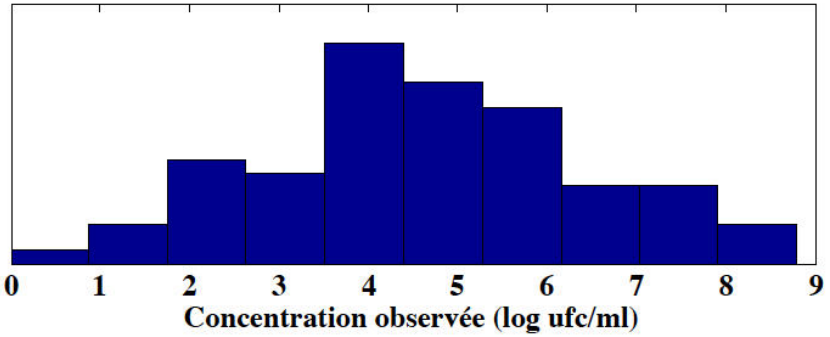
- Overestimation of contamination (mean: 6.2 log cfu/ml vs 4.6 log cfu/ml observed)

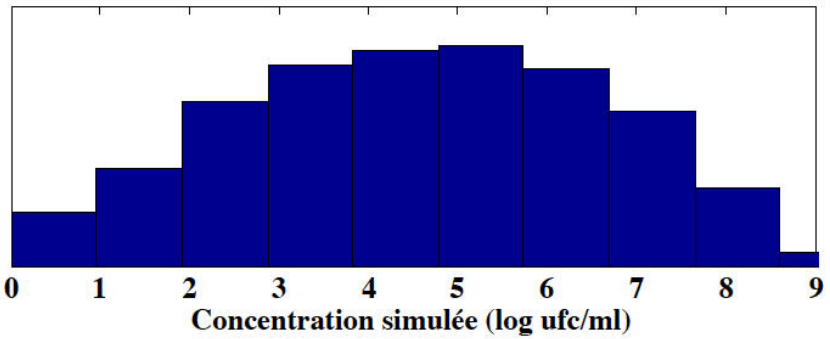
Modelisation of growth

- **Variability of Ci, growth rate (34 values), Nmax and individual cell lag time distributions** *(Dupont and Augustin 2009: influence of stress on single-cell lag time and growth probability for L. monocytogenes in half Fraser broth)*

- **Both observed and predicted concentrations have a mean of 4.6 log_{10} cfu/ml**







Modelisation of growth

- **Model validated for *L. monocytogenes* behaviour during half Fraser enrichment**

- **Use of the model to estimate loss of sensitivity in case of pooling of pre-enrichment broths**

Initial natural contamination

- **Initial contamination = Results of Baseline survey 2010-2011, for packaged hot or cold smoked or gravad fish at sampling**

Detection Testing	Enumeration testing: at least 10 cfu/g		
	Negative	Positive	Total
Negative	2 740	2	2 742
Positive	247	64	311
Total	2 987	66	3 053

	L. monocytogenes count (cfu/g)							
	< 10[(b)]	10-39	40-100	> 100-1 000	> 1 000-10 000	> 10 000-100 000	> 100 000	Total
Total No of samples	2 987	18	19	20	5	2	2	3 053

Use of the model to estimate *Lm* population after half-Fraser enrichment and loss of sensitivity in case of pooling of broths

- Enriched Half-Fraser or enriched Half- Fraser diluted 1/5
- Final concentration

Concentration (cfu/ml)	[0.004-10[	[10-10^2[	[10^2-10^3[	[10^3-10^4[	[10^4-10^6[	≥10^6
Single enrichment	18%	10%	15%	17%	27%	13%
Pooled enrichment	24%	14%	17%	16%	22%	7%

- Probability of detection

Detection threshold (cfu/ml)	10^2	10^3	10^4
Single enrichment	72%	57%	40%
Pooled enrichment	62%	45%	29%

> Loss of sensitivity ~ 10%

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- **Annex D: Verification protocol for pooling samples**

Use a standard suspension of an appropriately stressed strain (see ISO 16140-2) of the test microorganism appropriate to the method being investigated.

Inoculate test portions of the matrix at a level of approximately 5 cfu per 25 g (or ml)

The stress conditions applied should mimic the type of stress encountered by the target microorganism when present in a naturally contaminated sample of the product or environmental sample.

Verification protocol for pooling pre-enriched samples

- Enriched Half-Fraser or enriched Half-Fraser diluted 1/5
- Probability of detection

	Detection threshold (cfu/ml)	10^2	10^3	10^4
With stress	Single enrichment	46%	29%	16%
	Pooled enrichment	34%	19%	11%
Without stress	Single enrichment	94%	80%	52%
	Pooled enrichment	83%	61%	31%

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Power of the comparison

Detection probabilities to compare:	0.45 vs 0.33			1 vs 0.5	1 vs 0.7
Nb assays	5	10	200	5	9
Power	11%	14%	80%	80%	80%
Probability to see NO difference:	89%	86%	20%		

Conclusion and perspectives

- **10% loss of sensitivity of the detection method in case of wet pooling**
- **Is this loss acceptable for competent authorities?**
- **Balance advantages (improvement of sampling plans…) /disadvantages**
- **In case of wet pooling, may the specified ratio for sub-culturing step in Fraser be modified to 0,5 ml ?**
- **ISO/DIS 6887-1 annex D verification protocol allows to detect only high sensitivity loss (~50%) due to pooling**
  > Is acceptable loss the one defines by power of comparison of annex D verification protocol ?
- **Results of this study will be transferred to CEN/TC 275/WG 6/TAG 17 Listeria for the revision of EN ISO 11290-1, and may result in the addition of a note**

Thank you for your attention!