

Quantification of *Salmonella* spp.
using a miniaturisation of MSRV
enrichment medium :
mini-MSRV

Quantification of Salmonella spp.

Food safety :

- **retrospective studies of outbreaks** : could permit to rapidly identify the highly contaminated part of a control meal

Epidemiology

- identify the **at risk steps** in a primary production chain
- **reveal the efficiency** of partial decontamination procedure in highly contaminated environment

What exists?

- **Direct count** : Immunofluorescent or DNA probes
 - sensibility specificity antibodies (all serovars?)
 - technical steps (filtration...) micro-colonies
 - official method in AOAC and BAM (FDA)
- **Culture techniques** :
 - Direct on selective solid medium :
 - selectivity against dominant digestive flora
 - need selective or non selective concentration
 - selective media dedicated (Dulcitol-Bile-Novobiocine)
 - Culturability (stressed *Salmonella* cells) overlay procedure,
 - Most probable number methods...

Most probable number methods...

- Theory assumes two conditions :
 - organisms are randomly distributed throughout the solution
 - each sample from the solution which contains at least one organism is able to exhibit growth in the culture medium
- Both hypothesis are false (overall for Salmonella)
 - underline the need to well homogenised the solutions
 - select the most sensitive detection technique.

Most probable number methods...

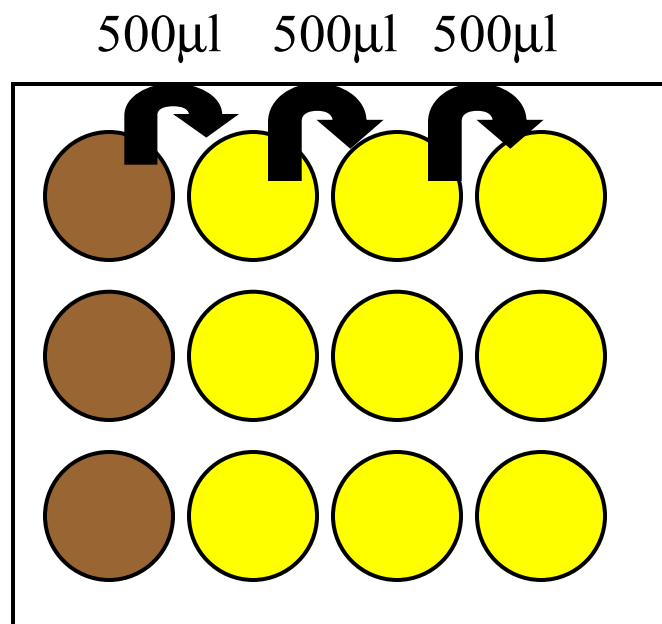
- Principe : Based on repetition of serial dilutions of a sample - Generate a characteristic number
- Calculation of the MPN :
 - using statistical assumption each characteristic number is related to a MPN with confidence limits
- Increase confidence of the result :
 - increase the number of repetition on the right dilution only if always almost the same level of contamination... so necessity to quantify?

Most probable number methods...

- Limits :
 - time and labor consuming
 - uncertain results
 - if low numbers, do we need quantification?
- Assuming this we propose
 - a rapid and compatible with high number of samples studies (at least 75 samples per week and person)
 - based on miniaturised MSRV enrichment
 - to discriminated highly from poorly contaminated samples

Mini-MSRV

- Sample diluted 1/10 in EPT : stomaching at least one minute



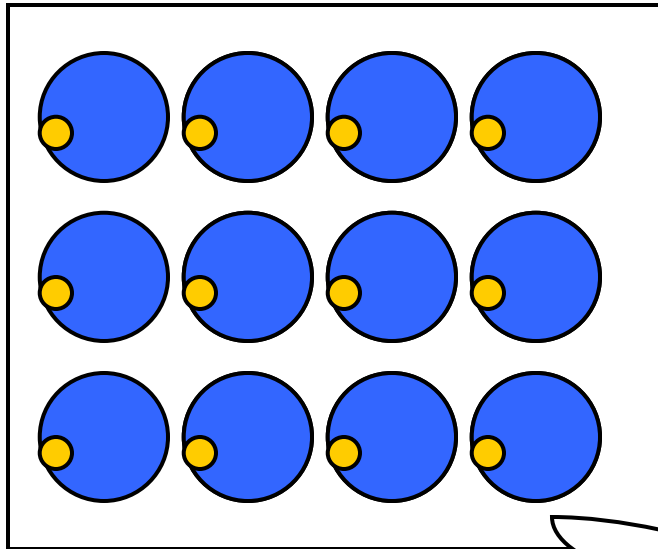
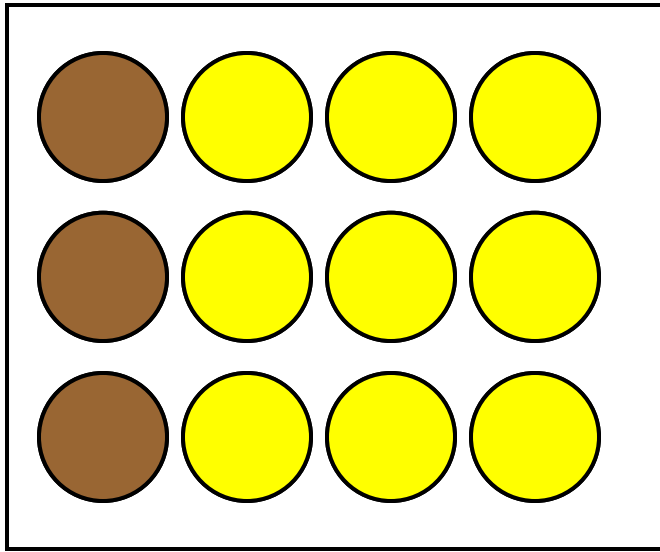
■ 2 mL EPT
■ 2,5 ml diluted sample

Serial dilution 1/5 with
multi-channel pipette

Incubation 16 h 37°C

Mini-MSRV

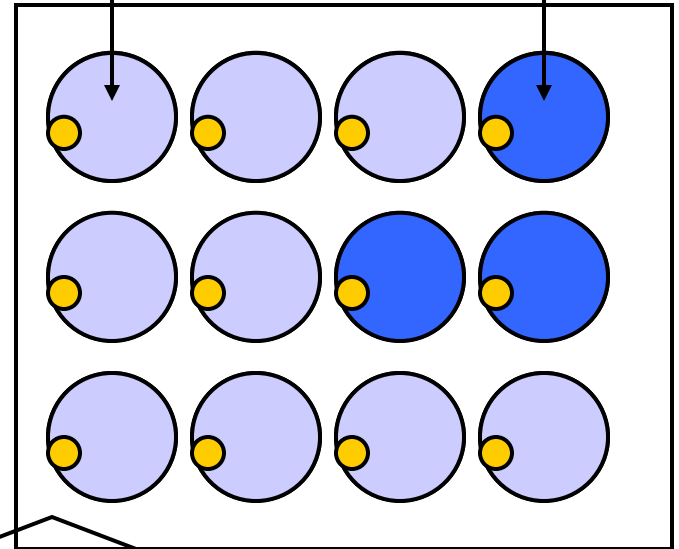
Replication
of the pre-enriched
plate 20 μ l/well
with multichannel pipette



Incubation
24 - 36h
41,5°C

Migration

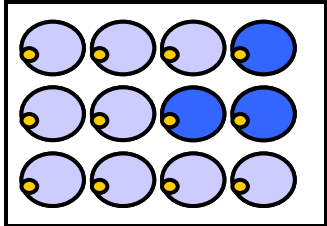
No migration



Mini-MSRV

- Streak each positive well on Rambach agar
- Biochemical characterisation of typical colonies
- Serogroup determination using polyspecific serums
- Determination of the characteristic number of the sample

Mini-MSRV



Characteristic number : 3321

<http://www.i2workout.com/mcuriale/mpn/index.html>

MPN deducted : $1,33 \cdot 10^2$ (IC : $4,9 \cdot 10^1 - 4,3 \cdot 10^2$)

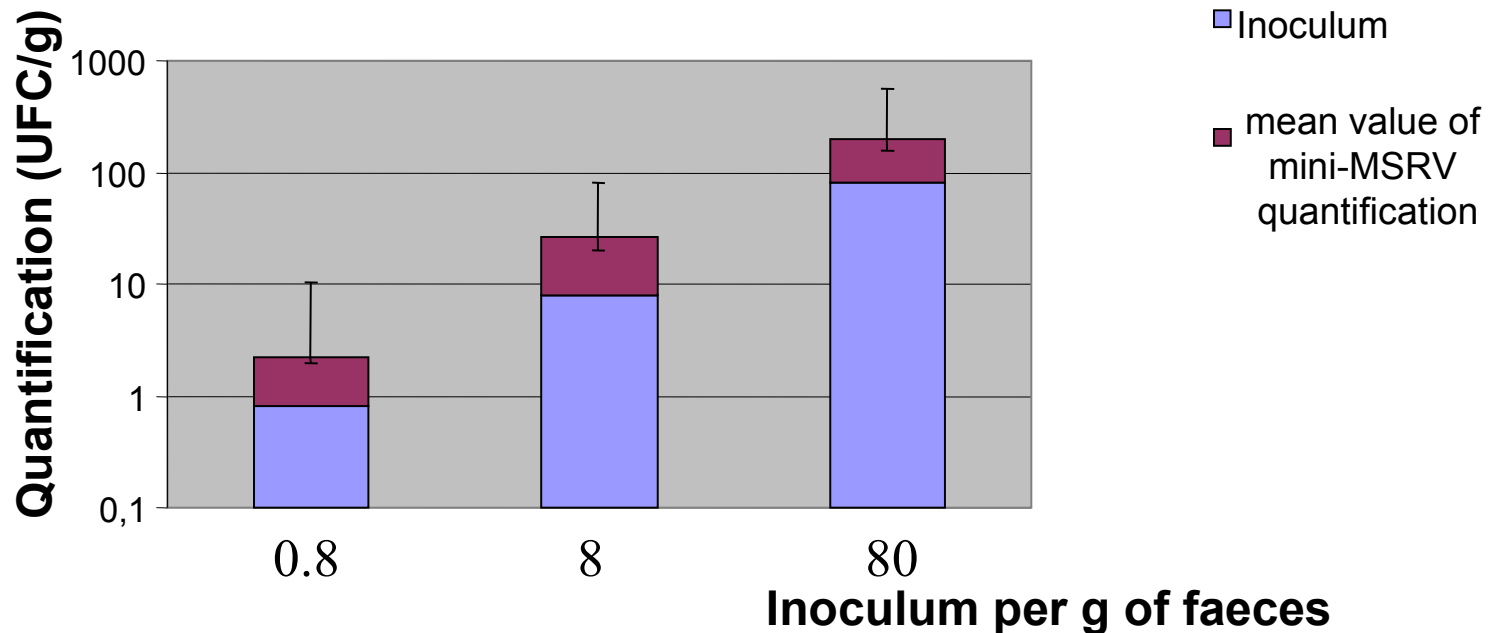
Salmonella per g of sample

(!Carefull to the initial dilution!)

	mini	Maxi
Characteristic nb	1000	3332
MPN (<i>Salmonella</i> /g)	1,2	$5,7 \cdot 10^2$
CI	0,17-8,65	$1,5 \cdot 10^2 - 2,1 \cdot 10^3$

Mini-MSRV

**Mean values obtained after mini-MSRV
quantification of artificially contaminated
pig faeces**




N = 7 samples independantly inoculated for each inoculum

Mini-MSRV Applications

- Quantification in pig fecal samples
- Quantification in surface samples in lairage
- Quantification in turkey neck skin samples

Mini-MSRV Applications

- Quantification in pig fecal samples

	Distribution of samples	<i>Salmonella</i> MPN per g	
	181	0,0	
	25	1,0	
	1	3,5	2 pigs populations
	4	6,4	
	4	$3,3 \cdot 10^1$	
	3	$1,8 \cdot 10^2$	
	6	$8,9 \cdot 10^3$	
TOTAL	224		

2 different risk levels for the production chain

Mini-MSRV Applications

- Quantification in surface samples in lairage
 - 300cm² per swab
 - 192 samples, 27 detected by mini-MSRV method
 - 24 positive samples present less than 1 *Salmonella* per cm² (MPN=0.82 IC 0.11 - 5.8)
- Improve the decontamination on homogeneised contaminated surfaces

Mini-MSRV Applications

- Quantification in turkey neck skin samples
 - samples from a positive herd
 - 10 g of neck skin
 - 122 samples, 14 positives, 9 detected by mini-MSRV method

Mini-MSRV Applications

Positive sample	MPN Salmonella /g of skin	IC	
1	0,5	1,4	5,4
2	0,1	0,6	4,2
3	0,1	0,6	4,2
4	1,4	3,9	10,7
5	0,1	0,5	4,2
6	1,3	3,5	9,8
7	0,7	2,1	6,9
8	3,1	8,3	21,9
9	0,1	0,6	4,2

Few numbers of bacteria on the skin of this suspected contaminated batch

Conclusion

- First investigations / well defined the need of quantification
- You could adapte the disposition of the plate or the number of plate fonction of the contamination level
- Convenient quantification method for high numbers of samples
- Development : more convenient if we need just to confirm the more diluted positive well