Challenges and misconceptions concerning pathogen testing in view of microbiological risk management

Marcel Zwietering
Laboratory of Food Microbiology

WAGENINGEN
UNIVERSITY & RESEARCH

1

Warning: Statistics is horrible !

- Complicated ……. Brrrr numbers
- Often giving very unpleasant messages
  - no zero risk
  - not significant
  - more data are needed

- That is all the same for sampling ……

WAGENINGEN
UNIVERSITY & RESEARCH

2

Absence….. Not Detected……. No control

- Low risk per serving is not low number of cases
- Low levels of pathogens do not mean no risk
- Low levels have statistically low probability to be detected
- Contamination can be very local
- Low levels need time to be detected

Do not reason away probabilities

WAGENINGEN UNIVERSITY & RESEARCH

3

Low risk per serving is not low number of cases

Examples of risk per serving of several diseases from RTE foods, risk per person per year, cases per year and cases per million population

Food product	Hazard	Region	Risk per serving	Risk per year per person	Cases per year	Cases/million population	Source
Deli meat	*L. monocytogenes*	USA[a]	$7.7 \cdot 10^{-8}$	$5.5 \cdot 10^{-6}$	1599	5.5	[23]
Unpasteurised milk	*L. monocytogenes*	USA[a]	$7.1 \cdot 10^{-9}$	$1.1 \cdot 10^{-8}$	3.1	0.011	[23]
Smoked seafood	*L. monocytogenes*	USA[a]	$6.27 \cdot 10^{-9}$	$4.5 \cdot 10^{-9}$	1.3	0.0045	[23]
Pasteurised milk	*L. monocytogenes*	USA[a]	$1.0 \cdot 10^{-9}$	$3.1 \cdot 10^{-7}$	90.8	0.31	[23]
Vegetables	*L. monocytogenes*	USA[a]	$2.8 \cdot 10^{-12}$	$6.9 \cdot 10^{-10}$	0.2	0.00069	[23]
Hard Cheese	*L. monocytogenes*	USA[a]	$4.5 \cdot 10^{-15}$	$1.4 \cdot 10^{-13}$	<0.1	<0.00035	[23]
Fermented meats	*L. monocytogenes*	Worldwide[b]	$2.5 \cdot 10^{-12}$	$6.6 \cdot 10^{-8}$	514.8	0.000066	[24]
Beef	*L. monocytogenes*	Brazil[c]	$8.1 \cdot 10^{-6}$	$1.2 \cdot 10^{-6}$	252	0.0000012	[25]
Beef	*Salmonella*	Brazil[c]	$4.7 \cdot 10^{-3}$	$8.6 \cdot 10^{-4}$	179,496	0.00086	[25]
Leafy green vegetable salad	*Salmonella*	The Netherlands[d]	$6.83 \cdot 10^{-6}$	$1.1 \cdot 10^{-5}$	187	10.82	[26]
Oysters	*Vibrio*	USA[a]	$4.5 \cdot 10^{-4}$ to $8.1 \cdot 10^{-1}$	$9.7 \cdot 10^{-6}$	2826	8.6	[27]
Oysters	*Vibrio*	Taiwan[e]	$8.56 \cdot 10^{-5}$	$2.8 \cdot 10^{-6}$	67	2.8	[28]
Shrimps	*Vibrio*	Malaysia[f]	$4.80 \cdot 10^{-6}$	$3.9 \cdot 10^{-6}$	123	12	[29]

WAGENINGEN UNIVERSITY & RESEARCH

All food processes have a residual risk, some are small, some very small and some are extremely small: zero risk does not exist

Marcel H Zwietering[1], Alberto Garre[1], Martin Wiedmann[2] and Robert L Buchanan[3,4]

4

We tested 5 samples and they were negative so the organism is absent !

- 100,000 chocolate bars of 25 g a day with 1 in 10,000 containing 1 *Salmonella*
- 5 samples of 25 g tested per day
- how many detects per year ?

WAGENINGEN UNIVERSITY & RESEARCH

5

But we tested 5 samples and they were negative so the organism is absent !

- 5 samples tested per day, 1 in 10,000 containing 1 *Salmonella*
- P_{detect}=5/10,000=0.0005 per day (1-(1-0.0001)^5)
- =0.1825 per year
- =1 detect every 5.5 years !
- so that is under control ?

WAGENINGEN UNIVERSITY & RESEARCH

6

low levels do not mean no risk

- 100,000 chocolate bars of 25 g a day with 1 in 10,000 containing 1 *Salmonella* = 10 *Salmonella* per day
- 10 per day is 3,650 *Salmonella* per year
- 1 *Salmonella* has 1:400 probability of illness (QMRA there is no MID !)
- 3,650/400=9.1 illness per year

- under control ? 9.1 cases ! "outbreak" ? can now be linked with WGS !

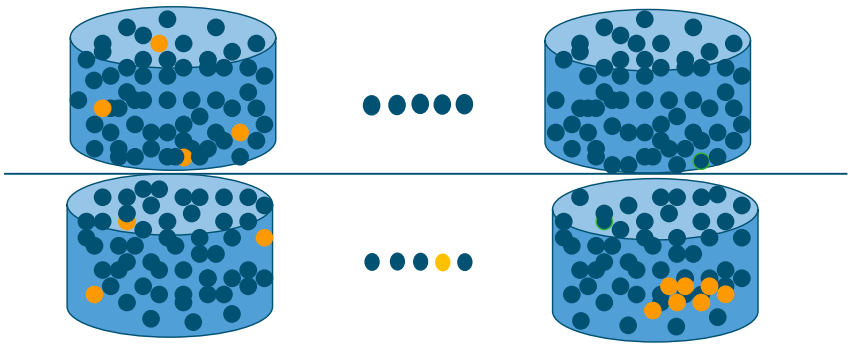
but risk per serving=9.1/36,500,000 = 1 per 4,000,000

1 per 77,000 per year (weekly bar)
1/900 with lifetime exposure (85 year LE)

WAGENINGEN UNIVERSITY & RESEARCH

7

End product testing useful or lottery ?



Positives mean something, negatives are no guarantee

MISCONCEPTION 1
If the tested sample units are negative, the batch is free of the pathogen.

WAGENINGEN UNIVERSITY & RESEARCH

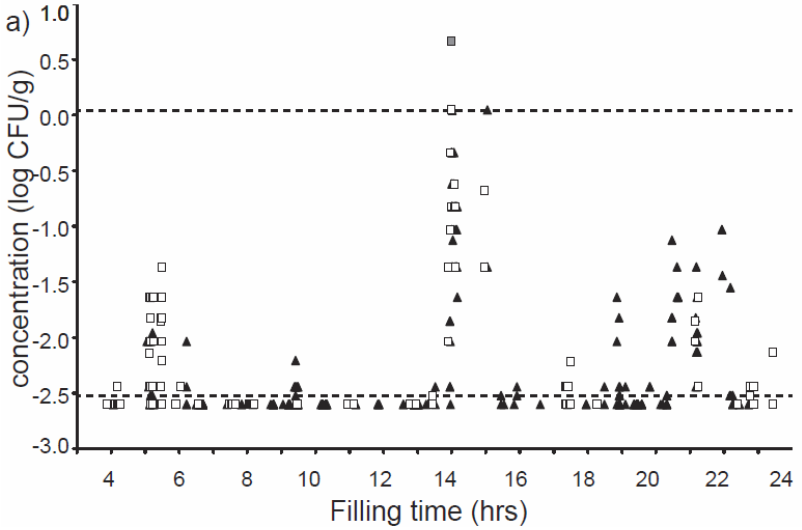
8

Contamination can be very local

- Recalled batch: no positives in 10*10 g Enteros
- Later detected by a government
- 415 MPN's:
  3*100 g; 3*10 g; 3*1 g
  =138 kg !

70% chance to detect…..
30% chance not to find it

Now (30*10) 97 % to detect
3% to miss



Actual distribution of *Cronobacter* spp. in industrial batches of powdered infant formula and consequences for performance of sampling strategies. I. Jongenburger, M.W. Reij, E.P.J. Boer, L.G.M. Gorris, M.H. Zwietering (2011)

WAGENINGEN UNIVERSITY & RESEARCH

9

Not detected is no guarantee

- Low risk per serving is not low number of cases
- Low levels of pathogens do not mean no risk
- Low levels have statistically low probability to be detected
- Contamination can be very local

- Often the levels we can test for are not levels we should have

WAGENINGEN UNIVERSITY & RESEARCH

10

Low levels need time to be detected

- New methods are faster and have higher specificity/sensitivity ?
- They are faster in part of the procedure
- They are very "specific" in the final identification but not at all "sensitive"
- First enrichment is needed, and if that is "forgotten" in the marketing of methods this is not really right

WAGENINGEN UNIVERSITY & RESEARCH

11

Criteria for *Salmonella*, EHEC, *Listeria Campylobacter* are 1 cell in 25 g !

During EHEC outbreak in sprouts in 2011 some researchers in the NL were marketing a method to detect EHEC in 15 min. They spiked the vegetables with 10^9 cells and indeed could detect the organism in 15 min……

This is making conventional methods look foolish, why wait so long ?

But this is not fair at all

WAGENINGEN UNIVERSITY & RESEARCH

12

Criteria for *Salmonella*, EHEC, *Listeria Campylobacter* are 1 cell in 25 g !

- 1 cell in 25 gram = 1 cell 250 ml = 0.004 cfu/ml
- Factor 25,000 lower than 10^2 cfu/ml the detection limit of many methods

Enrichment is critical in a reliable detection

You CANNOT prove absence in 250 ml in a vial of 100µl

WAGENINGEN UNIVERSITY & RESEARCH

13

Lower than a needle in a haystack

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Food Science

Review

Sampling and testing for pathogens in food: finding the needle in a haystack and the impact of the food microbiome

Heidy MW den Besten, Johanna Mentani and Marcel H Zwietering

WAGENINGEN UNIVERSITY & RESEARCH

14

really lower than a needle in a haystack

- bacterial cell: cylinder of 3 µm by 1.5 µm, its volume will be

$V_{cell} = \pi \times (0.75\ \mu m)^2 \cdot 3\ \mu m \sim 5\ \mu m^3 = 5 \cdot 10^{-18}\ m^3 = 5 \cdot 10^{-12}$ ml

Volume fraction one cell in 25 ml: $5 \cdot 10^{-12}$ ml/25 ml = $2 \cdot 10^{-13}$ ml bacterium/ml food

This is 1 part per $5 \cdot 10^{12}$ parts i.e., 0.2 ppt on volume basis (or 200 ppq)

- A needle is a cylinder of 5 cm by 0.5 mm, its volume will be

$V_{needle} = \pi \times (0.25\ mm)^2 \cdot 50\ mm = 10\ mm^3 = 1 \cdot 10^{-8}\ m^3$

When we assume the haystack to be 1000 m^3, the needle is 1 part per $1 \cdot 10^{11}$ parts

- So to find one pathogen in 25 ml of food is like finding a needle in 50 haystacks

WAGENINGEN UNIVERSITY & RESEARCH

million (10^{-6}) billion (10^{-9}) trillion (10^{-12}) quadrillion (10^{-15})

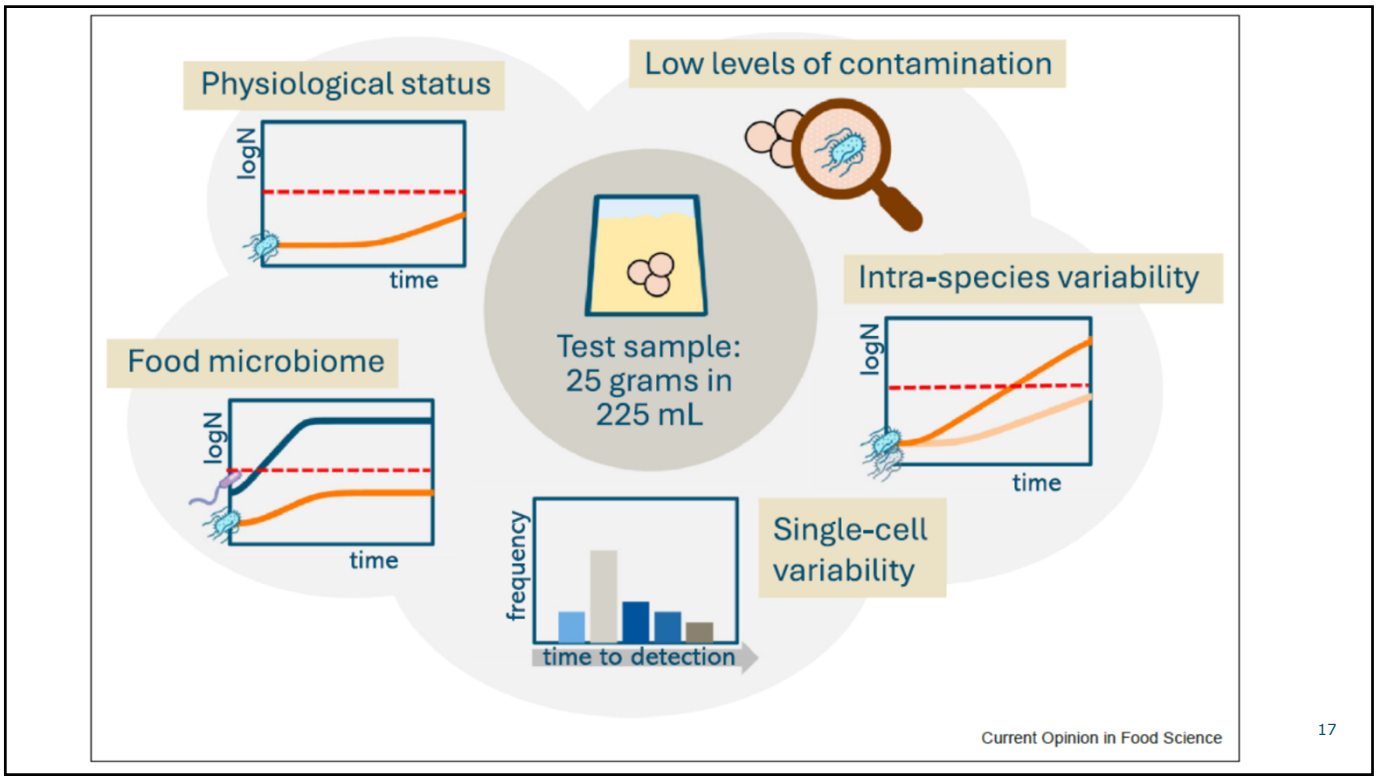
15

really lower than a needle in a haystack

- For a needle you use a magnet, but still a lot of work
- For bacteria you can use PCR, gene copies, RNA copies, filtering, magnetic beads…. it all helps, but you still need to go through your 250 ml !

WAGENINGEN UNIVERSITY & RESEARCH

16

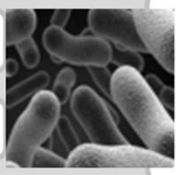


17

Enrichment needed !

damage repair

amplification



(selective) enrichment

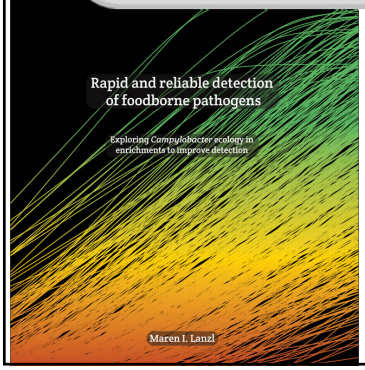
detection and identification

10^2-10^4 cfu/ml

molecular-based

culturing-based

10^2 cfu/ml



low levels
damaged cells
competitive biota

18

Not detected is no guarantee

- Low risk per serving is not low number of cases
- Low levels of pathogens do not mean no risk
- Low levels have statistically low probability to be detected
- Contamination can be very local
- Low levels need time to be detected
  - time
  - recovery
  - background
  - media (selectivity)

WAGENINGEN UNIVERSITY & RESEARCH

19

Conclusions: Do not reason away probabilities

- Low risk per serving can still give many cases
- Low levels do not mean no risk
- Low levels have low probability to be detected
- Local contamination even more difficult to find
- Low levels need time to be detected (stress / background biota)

WAGENINGEN UNIVERSITY & RESEARCH

20