



Preliminary results EURL-*Salmonella* Proficiency Test food- feed 2019

Detection of *Salmonella* in
flaxseed

Robin Diddens



EURL-*Salmonella* PT food-feed 2019

- Problems PT feed 2018
 - Not possible to evaluate performance of NRLs
- Combined food-feed PT
- Ideas: corn flour, flaxseed, wheat, oats
- For human consumption and chicken feed



Ideas: corn flour, flaxseed, wheat, oats

- Practical in the laboratory?
- Natural background flora?
- Winner: broken flaxseed
 - Easy to handle in the laboratory
 - High concentration *Enterobacteriaceae* and aerobic count



Assessment of the microbiological safety of edible dried seeds from retail premises in the United Kingdom with a focus on *Salmonella* spp.

Caroline Willis ^a, Christine L. Little ^b, Satnam Sagoo ^b, Elizabeth de Pinna ^b, John Threlfall ^b

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<https://doi.org/10.1016/j.fm.2009.05.007>

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Abstract

Sesame seed products have recently been associated with a number of *Salmonella* outbreaks in the UK and elsewhere. Aside from sesame seeds, there is little published information on the prevalence of *Salmonella* spp. in edible seeds. A study of 3735 samples of retail edible dried seeds in the UK was therefore carried out between October 2007 and March 2008 to assess their microbiological safety in relation to *Salmonella* contamination and levels of *Escherichia coli*, an indicator of faecal contamination. Overall, *Salmonella* was detected in 23 samples (0.6%), of which over half (57%) were sesame seeds. Other seeds contaminated with *Salmonella* were linseed (1 sample), sunflower (1 sample), alfalfa (1 sample), melon (4 samples) and mixed seeds (3 samples). *E. coli* was detected in 9% of samples, with 1.5% containing unsatisfactory levels ($\geq 10^2$ /g). These included melon, pumpkin, sesame, hemp, poppy, linseed, sunflower and mixed seeds. The UK retailers affected by the

in chia and flax

Salmonella-in-chia-and-flax-seed-powder-

Monday recalled specific lots of R and Organic traditions they have the potential to be a danger to human health.

Random samples of the recalled products were taken by Canadian Food Inspection Agency (CFIA) from retail stores in Canada and subsequently tested positive for Salmonella. CFIA then notified the supplier. Health Matters America Inc.

eus

ut food serious Details

Multi-country outbreak associated with

Salmonella enterica, serotype Enteritidis
Genome Sequencing (WGS)

First published in EFSA

Approved: 12 June 2009

Type: Technical Report



Abstract

An outbreak of a new serotype of salmonella with an antigenic formula 11:z4:1 individuals in five European countries: Luxembourg and the United Kingdom



Flaxseed



Flax fibers (linen)
Flaxseed oil
Flaxseed





Flaxseed





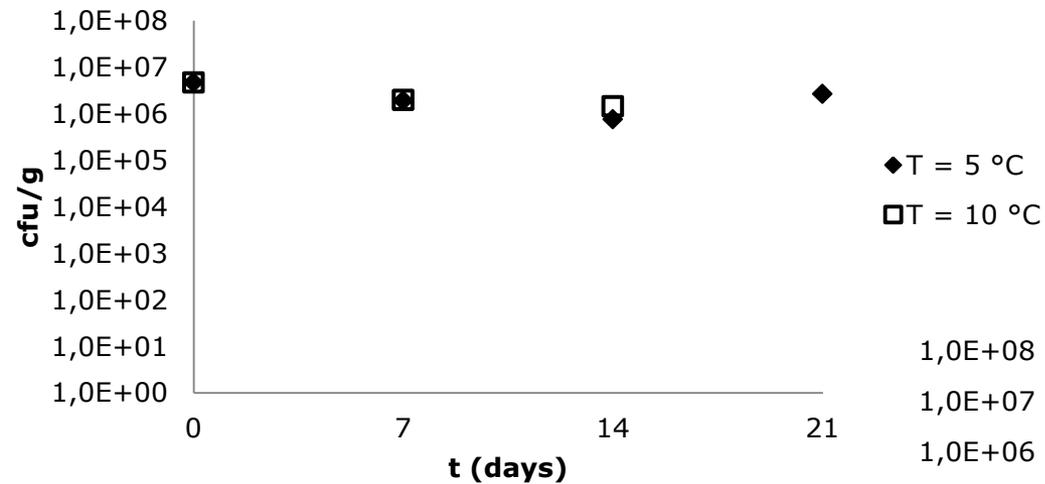
Pre-test

- *Salmonella* free flaxseed
 - 35.5 kg batch – 10 samples tested
- 3 different concentrations of *S. Typhimurium*
 - 6 cfu / sample
 - 10 cfu / sample
 - 24 cfu / sample
- Storage temperatures: 5°C and 10°C
- Testing at t = 0, 7, 14 and 21 days
- 6 samples per conc. STm, storage T and testing day
- Test of natural background flora
 - *Enterobacteriaceae* & aerobic count

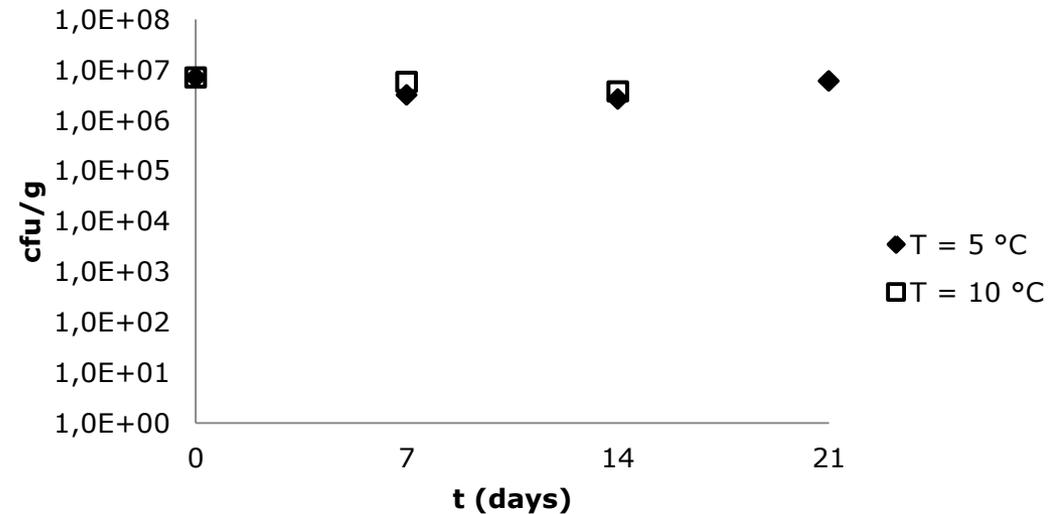


Pre-test – Natural background flora

Flaxseed - *Enterobacteriaceae*



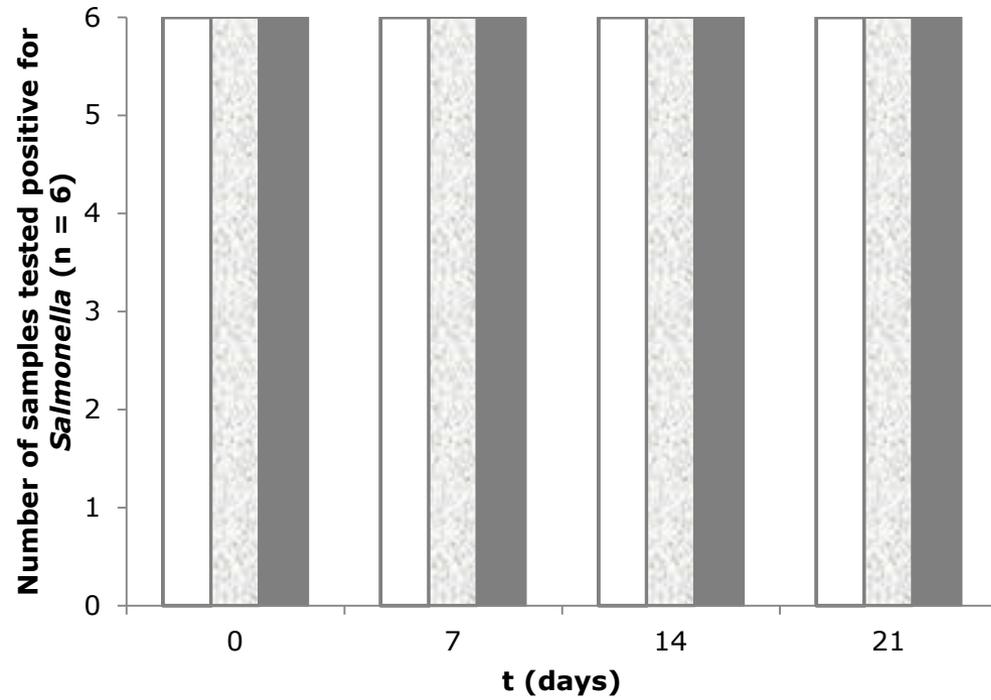
Flaxseed - aerobic count





Pre-test - *Salmonella*

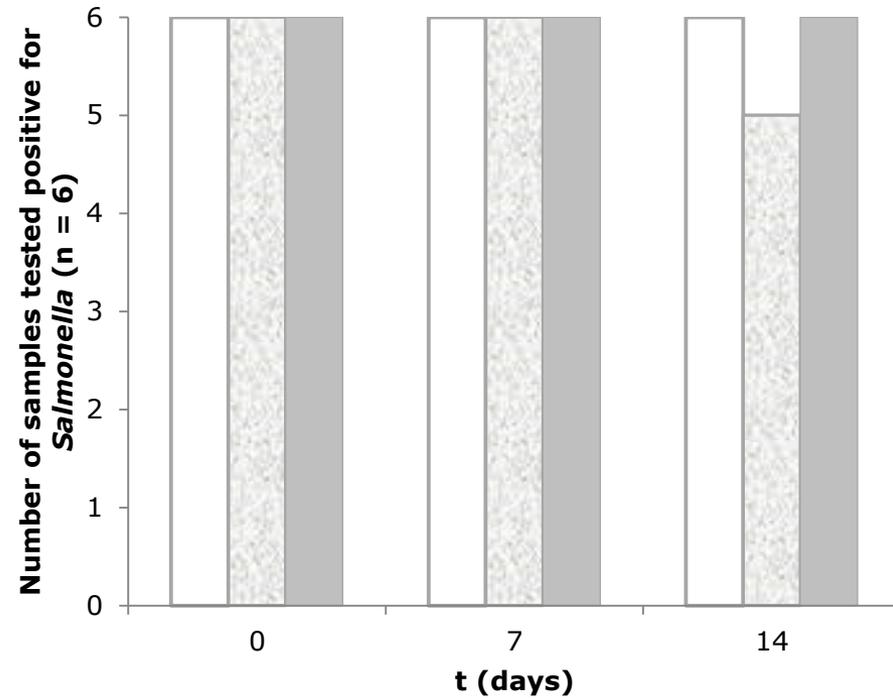
5 Celsius



□ 6 cfu / 25 g flaxseed
■ 24 cfu / 25 g flaxseed

□ 10 cfu / 25 g flaxseed

10 Celsius



□ 6 cfu / 25 g flaxseed
■ 24 cfu / 25 g flaxseed

□ 10 cfu / 25 g flaxseed



Proficiency Test design

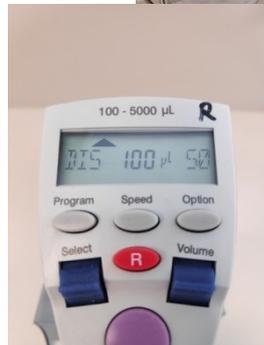
- Samples: 25 g flaxseed
- *Salmonella* Typhimurium ATCC 14028

- 18 samples (B1 – B18):
 - 6 negative samples (no *Salmonella* added)
 - 6 samples with a low level of *Salmonella* Typhimurium
 - > 5 – 10 cfu / 25 g flaxseed
 - 6 samples with a high level of *Salmonella* Typhimurium
 - > 10 x concentration low level: 50 – 100 cfu / 25 g flaxseed

- 2 control samples
 - Sample C1: only BPW
 - Sample C2: own positive control (with or without matrix)



Preparation of flaxseed samples





Preparation of flaxseed samples





Preparation of the parcel





Participants

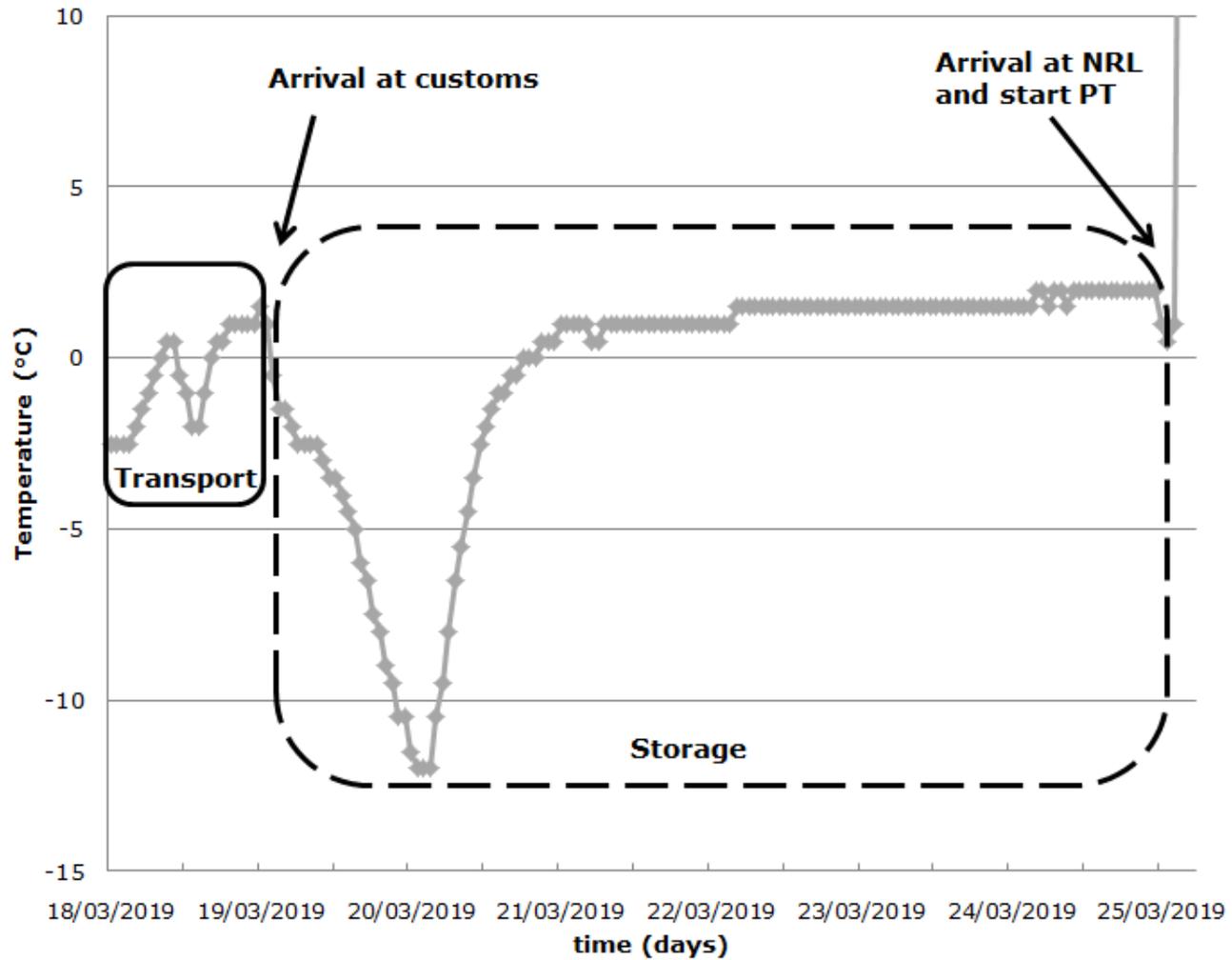


- 42 laboratories participated
 - 28 food & animal feed labs
 - 8 food labs
 - 6 animal feed labs
- 37 NRLs from 28 EU-Member States (MS)
- 5 NRLs from EU candidate MS and members of the European Free Trade Association (EFTA)
- 1 NRL from potential candidate MS problems receiving parcel



Temperature during transport and storage

- 41 parcels arrived within 1 to 2 days at the NRLs
 - Transport below 5 °C
 - Storage
 - > 38 NRLs at below 7.5 °C
 - > 3 NRLs temperature measured of 9.5 °C - 11.5 °C
- 1 parcel arrived after 7 days at NRL





Method(s)

- Prescribed method
 - EN ISO 6887-4:2017
 - › Protocol for the preparation of samples
 - EN ISO 6579-1:2017
 - › BPW
 - › Selective enrichment: MKTTn & RVS and/or MSRV
 - › Selective isolation media: XLD and second choice
 - › Confirmation
- Second detection method
 - Not used to assess performance

**Microbiology of the food chain —
Horizontal method for the detection,
enumeration and serotyping of
Salmonella —**

Part 1:
Detection of *Salmonella* spp.

*Microbiologie de la chaîne alimentaire — Méthode horizontale
pour la recherche, le dénombrement et le sérotypage des
Salmonella —*

*Partie 1: Recherche des *Salmonella* spp.*



Inoculum

Date of testing	Low level STm in cfu per sample	High level STm in cfu per sample
12 March 2019 Inoculation of flaxseed	10	105
25 March 2019 ^a MPN of flaxseed, inoculated with STm (95% confidence limit)	13 (4.5-37.5)	160 (52.5-500)

a. After storage at 5 °C for 2 weeks



Natural background flora flaxseed

Date of testing	<i>Enterobacteriaceae</i> (cfu/g)	Aerobic bacteria (cfu/g)
21 November 2018	4.6×10^6	7.0×10^6
25 March 2019 ^a	4.0×10^5	1.6×10^6

a. After storage at room temperature for 4 months and at 5 °C for 2 weeks

Enterobacteriaceae: EN ISO 21528-2:2017

Aerobic bacteria: EN ISO 4833-1:2013



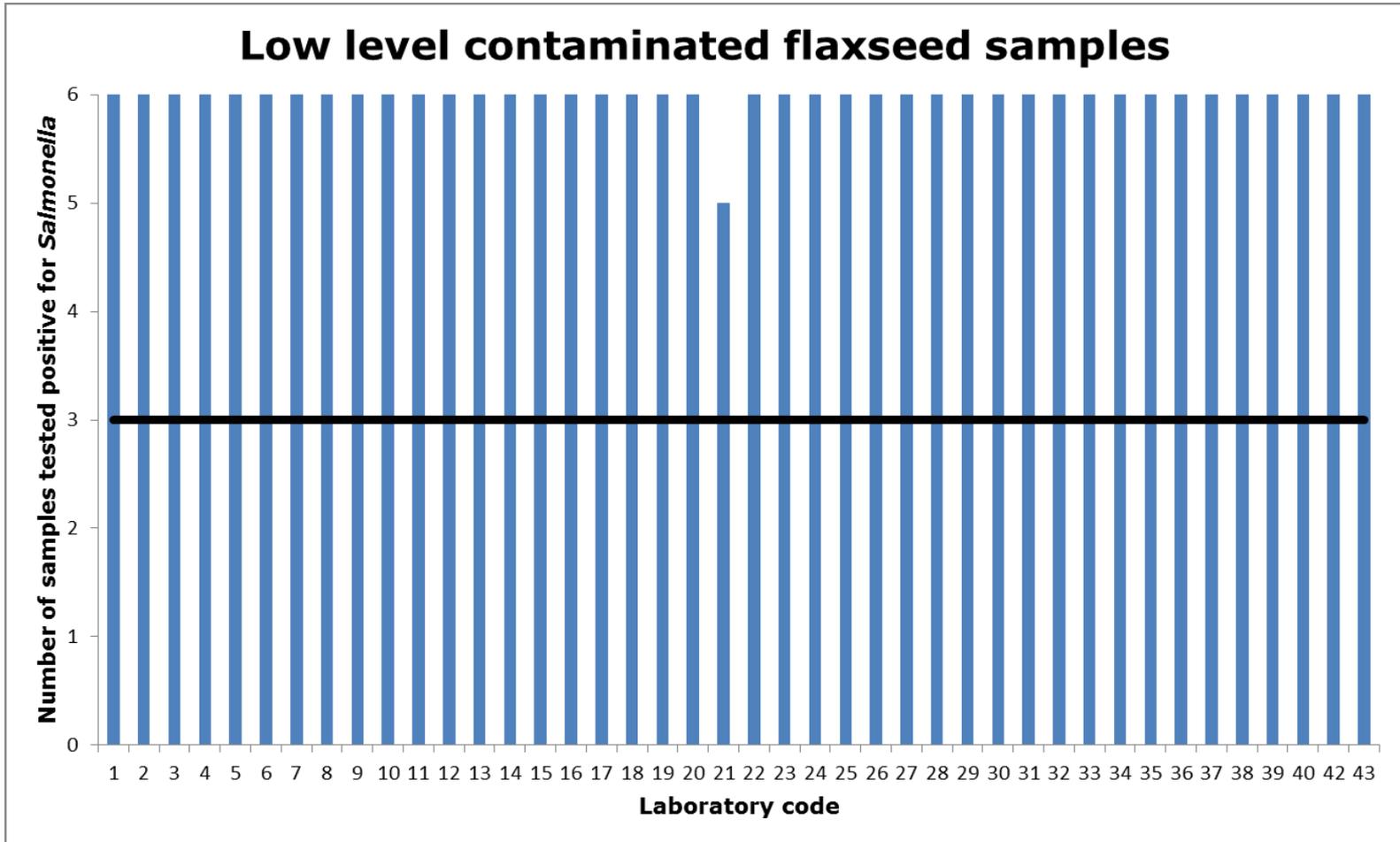
Criteria for good performance

Contaminated samples	Percentage positive	# pos samples/ total # samples
Negative samples*	20% max	1/6 max
Low level contamination	50%	3/6
High level contamination	80%	5/6
Control samples	Percentage positive	# pos samples/ total # samples
BPW	0%	0/1
Own positive control	100%	1/1

*100% *Salmonella* free matrix cannot be guaranteed, 1 positive out of 6 negative samples is still considered as acceptable (20%)



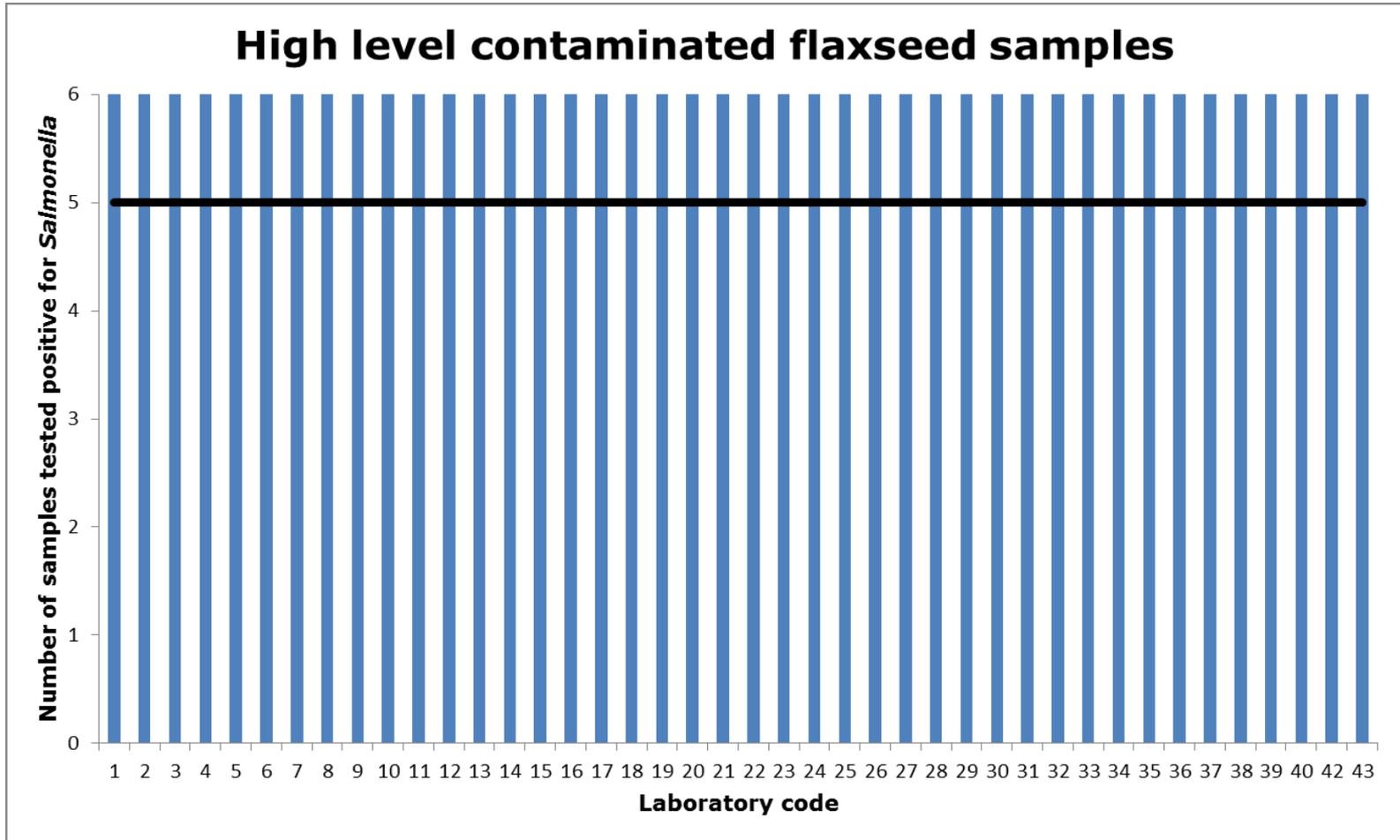
Results



— Criteria for good performance



Results



Criteria for good performance



Specificity, sensitivity & accuracy

Flaxseed	Percentage positive	Total laboratories n = 42
Negative samples n = 6	No. of samples	252
	No. of negative samples	251
	Specificity in %	99.6%

Laboratory 42 detected *Salmonella* in a negative sample, but made an error in reporting the results

All flaxseed samples artificially contaminated with <i>Salmonella</i>	No. of samples	504
	No. of positive samples	503
	Sensitivity in %	99.8%
All flaxseed samples	No. of samples	756
	No. of positive samples	754
	Accuracy in %	99.7%



Specificity, sensitivity & accuracy

Control samples	Percentage positive	Total laboratories n = 42
BPW	No. of samples	42
	No. of negative samples	41
	Correct score in %	97.6%
Own positive control	No. of samples	42
	No. of negative samples	41
	Correct score in %	97.6%

Laboratory 13 incorrectly reported the control samples
Moderate performance



All other laboratories
fulfilled the criteria of
good performance



Second detection method

- Used (routinely) in laboratories to analyse samples
- 7 NRLs-*Salmonella* used qPCR
- 5 NRLs-*Salmonella* used PCR
- 1 NRL-*Salmonella* used mini vidas
- All validated methods
- Similar results to the prescribed method EN ISO 6579-1:2017



Technical deviations

Laboratory code	hours incubation BPW	MKTTn			RVS		MSRV		
		concentration novobiocin (mg /L)	pH	Temperature (°C)	pH	Temperature (°C)	concentration novobiocin (mg/L)	pH	Temperature (°C)
EN ISO 6579-1	18 ± 2 hours	40 mg /L	7 - 8.2	37.0 °C ± 1 °C	5.2 ± 0.2	41.5 °C ± 1 °C	10 mg / L	5.1 - 5.4	41.5 °C ± 1 °C
1	18						10	5.2	41.5
2	18	?	6.9	37	5.3	42			
5	22	39	7.05	37	5.30	41.4			
6	18	40	8.00	37	5.25	41.5	50	5.25	41.5
9	18				5.25	42	10	5.5	42
12	18	40	8.0	37			20	5.4	37
13	18	40	8.1	36.93	8.3	36.93	?	8.1	36.93
15	24	40	not measured	37	not measured	41.5			
17	20	40	6.6	37	5.2	41.5			
20	19	40	8.0	37			10	5.5	41.5
23	15	40	8.0 +/- 0.2	37.0	5.2 +/- 0.2	41.0	10	5.2 +/- 0.2	41.0
24	20						20	5.3	41.5
29	18	5	8.04	37	5.23	41.5	10	5.61	41.5
34	19	0	7.80	41.2	5.25	41.2			
36	18	10	8	37			10	5.2	42
37	18	40	8.06	37			10	5.48	41.5
38	18	40	5.2	37	8.1	41.5			
40	20	40	7.99	37	5.30	41.5	10	5.60	41.5
42	18	40	7.9	37			10	5.6	41.5



Results – Positive control

- Concentration positive control: $2 - 10^8$ cfu / sample
 - 2 - 120 cfu / sample (29x)
 - 121 - 9.99×10^2 cfu / sample (5x)
 - $10^3 - 10^8$ cfu / sample (6x)
 - Not known (2x)
- Serovars used:
 - *S. Enteritidis* (15), *S. Typhimurium* (10), *S. Nottingham* (7), and 10 participants used other *Salmonella* serovars
- 5 laboratories added matrix to own positive control
 - (chia seeds, fishmeal, food, meat, minced meat)



THANK YOU!

Questions?

