



National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Preliminary results EURL-*Salmonella* Proficiency Test food-feed 2023

Detection of *Salmonella* spp. in
flaxseed

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EURL-*Salmonella* workshop May 2023



EURL-*Salmonella* PT food-feed 2023

- › Combined food and feed Proficiency Test

- › In 2019 also a combined PT

- › Matrix: flaxseed
 - For human consumption and chicken feed
 - Practical to work
 - High concentration aerobic count and *Enterobacteriaceae*
 - *Salmonella* stable in samples



Flaxseed

Flax is a flowering plant

- Flax fibers are used for linen
- Flaxseed oil
 - Flaxseed pallets
- Flaxseed

Salmonella

JOINT ECDC-EFSA RAPID OUTBREAK ASSESSMENT

Multi-country outbreak of multiple *Salmonella enterica* serotypes linked to imported sesame-based products

14 October 2021

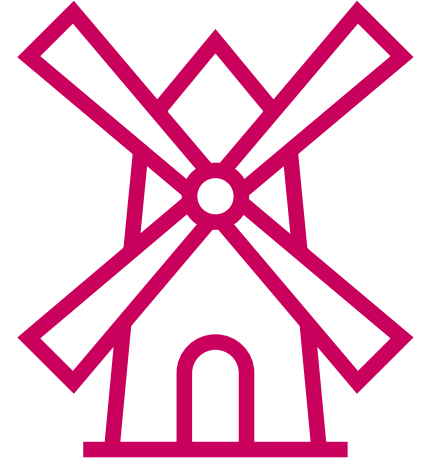
Abstract

Since January 2019, 121 cases of *Salmonella enterica* infections with six different serotypes linked to sesame-based products have been reported in five EU/EEA countries. The serotypes are *S. Amsterdam*, *S. Havana*, *S. Kintambo*, *S. Mbandaka*, *S. Orion*, and *S. Senftenberg*. Case interviews in four countries revealed consumption of sesame-based products (halva or tahini) prior to illness. Almost half of the cases are in children ≤ 10 years, who also represent over half of hospitalised cases. No deaths have been reported.



PT food-feed 2023 - Pre-tests

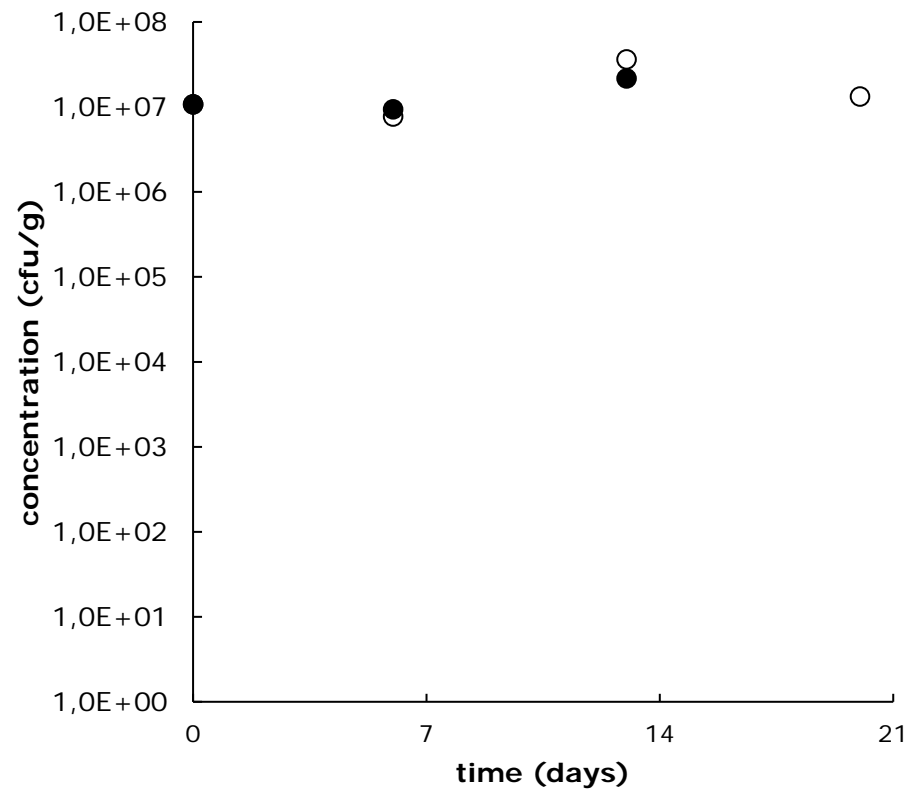
- › 25 kg batch (broken) flaxseed
 - 10 samples tested: *Salmonella* free
- › Test of natural background flora
 - aerobic count & *Enterobacteriaceae*
- › Spike with different concentrations *S. Typhimurium*
 - 7 cfu/sample and 11 cfu/sample
- › Storage temperatures: 5 °C and 10 °C
- › Testing at t = 0, 7, 14 (and 21 days)
- › 6 samples per conc. STm, storage T and testing day



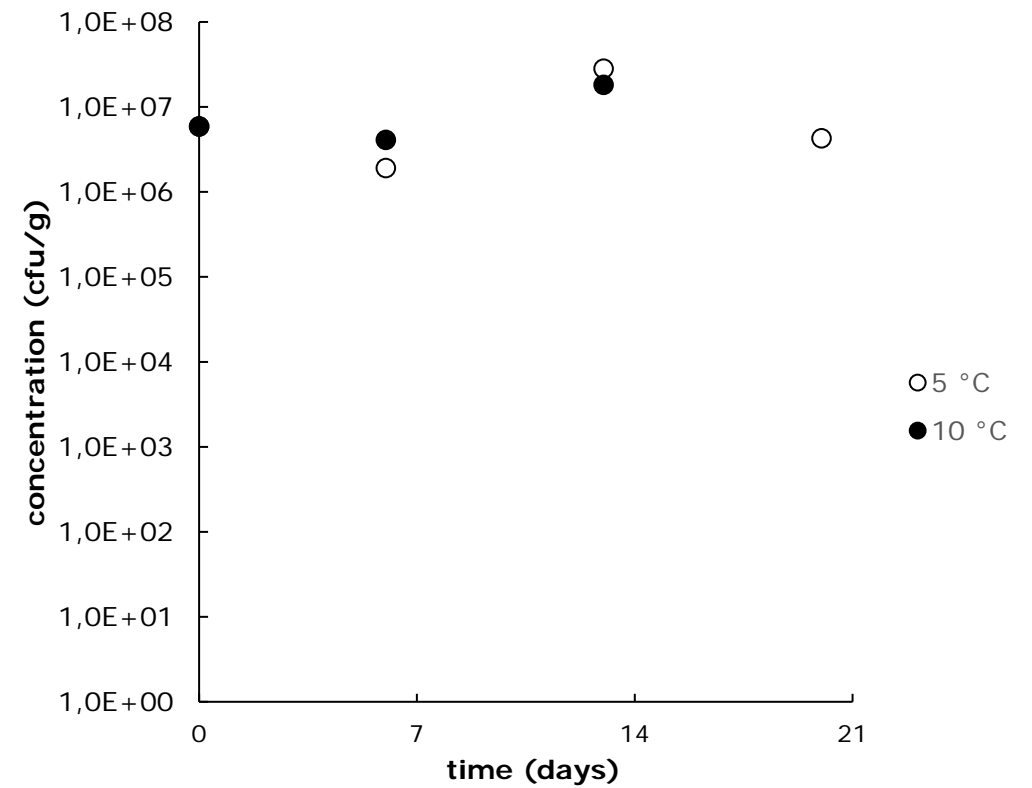


Pre-test background flora

Aerobic count



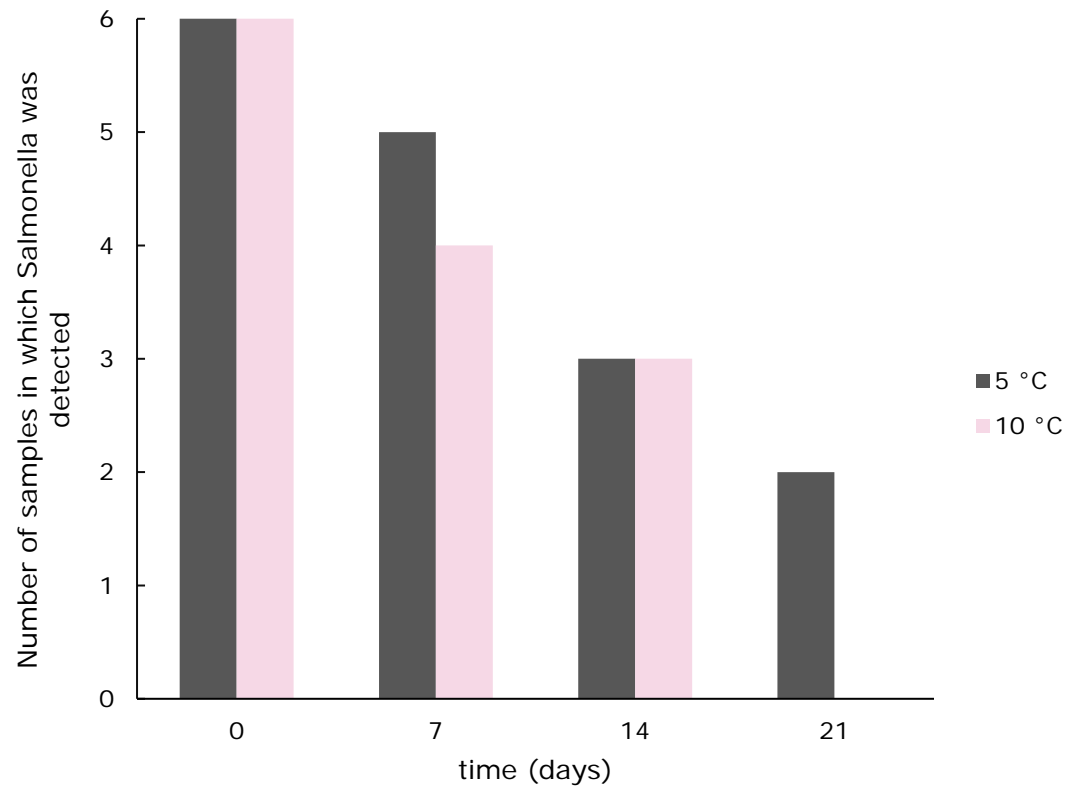
Enterobacteriaceae



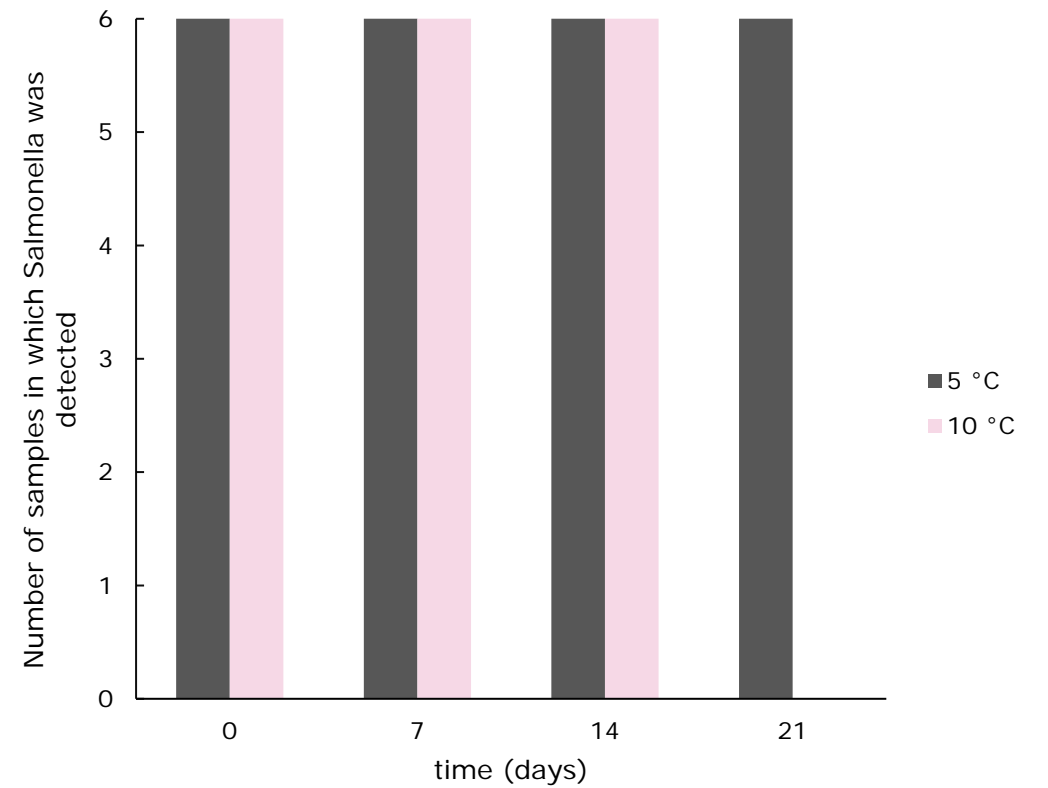


Pre-test *Salmonella*

Spike: 7 cfu/sample



Spike: 11 cfu/sample





Proficiency Test design

- > 25 g flaxseed samples
- > *S. Typhimurium* (ATTC 14028)

- > 14 samples (A1-A14)
 - 4 negative samples
 - no *Salmonella* added
 - 6 low level samples
 - aim 9 cfu/sample
 - 4 high level samples
 - aim 50 cfu/sample

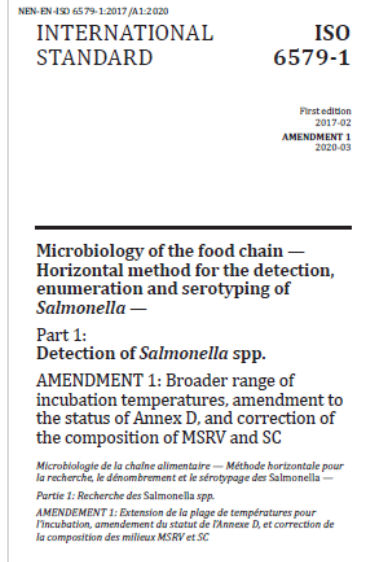
- > 2 control samples
 - CTRL1: Procedure control (BPW only)
 - CTRL2: Positive control (own *Salmonella* control)
 - The control samples can be performed either way, but reporting of the results should be as described



Methods

- > Preparation of the samples
 - EN ISO 6887-4:2017
 - Adjusted protocol
 - 1. Add the BPW to the 25 g test sample (instead of weighing accurately the sample into a pre-dispense volume of BPW);
 - 2. Resuscitate the sample for 20 to 30 minutes at 18 °C to 27 °C (room temperature);
 - 3. Mix for 30 seconds (\pm 5 seconds) by hand;
 - 4. Continue with the non-selective pre-enrichment procedure as described in EN ISO 6579-1:2017(/A1:2020).

- > Analysing of the samples
 - EN ISO 6579-1:2017(/Amd 1:2020)
 - BPW
 - Selective enrichment: MKTTn & RVS and/or MSRV
 - Selective isolation media: XLD and second choice
 - Confirmation
- > Second detection method
 - Not used to assess performance





Participants

- › 51 participants
 - 26 NRLs food-feed
 - Multiple laboratory codes and sets of samples
 - 7 NRLs food
 - 7 NRLs feed
 - 1 NRL vegetal matrices

- › NRLs from 27 EU Member States

- › 6 NRLs from third countries
 - EU candidate MS, members of the European Free Trade Association (EFTA), and United Kingdom



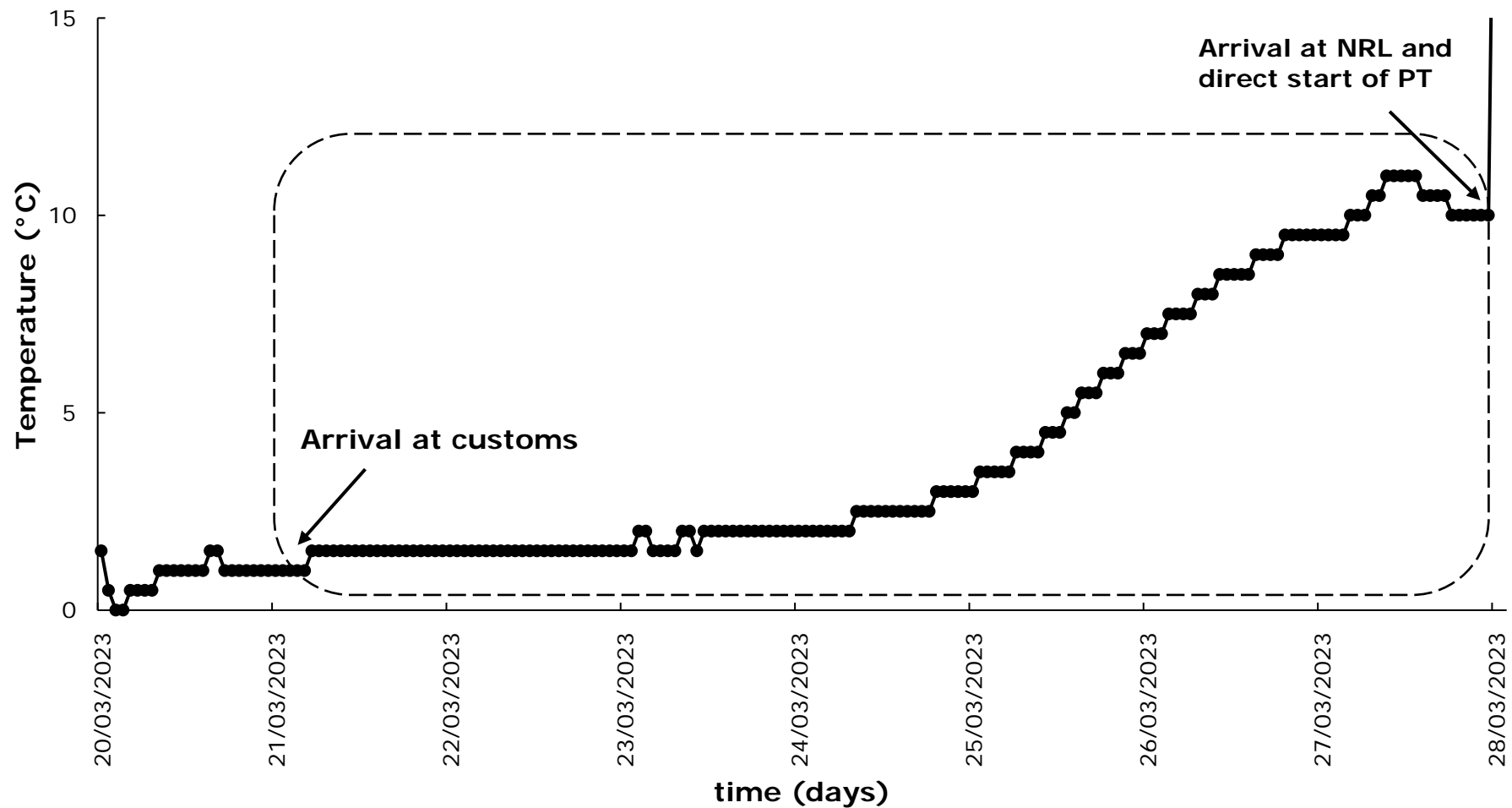


Temperature during transport and storage

- › 40 parcels were delivered within 1 to 2 days at the NRLs
- › Temperature measured with button
 - Transport below 2,5 °C
 - Storage between 0 °C and 9 °C
- › 1 parcel was delivered after 8 days of transport
 - Held at customs



Temperature during transport and storage



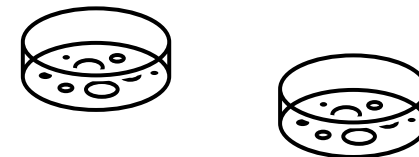


Natural background flora of the PT samples

Date	Aerobic bacteria (cfu/g)	<i>Enterobacteriaceae</i> (cfu/g)
10 January 2023	$1,1 \times 10^7$	$5,9 \times 10^6$
27 March 2023 ^a	$4,0 \times 10^6$	$2,1 \times 10^6$

a. After storage at room temperature for 9 weeks and at 5 °C for 13 days

Aerobic bacteria: EN ISO 4833-1:2013
Enterobacteriaceae: EN ISO 21528-2:2017

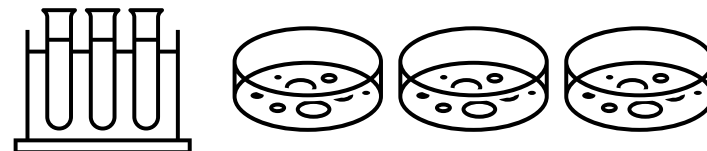




Artificially contamination

Date	Low level STm in cfu per sample	High level STm in cfu per sample
14 March 2023 Inoculation of flaxseed samples	9	52
27 March 2023 ^a MPN of flaxseed samples, inoculated with STm (95% confidence limit)	3,25 (1,1-10,3)	7 (2,3-21,5)

a. After storage at 5 °C for 13 days





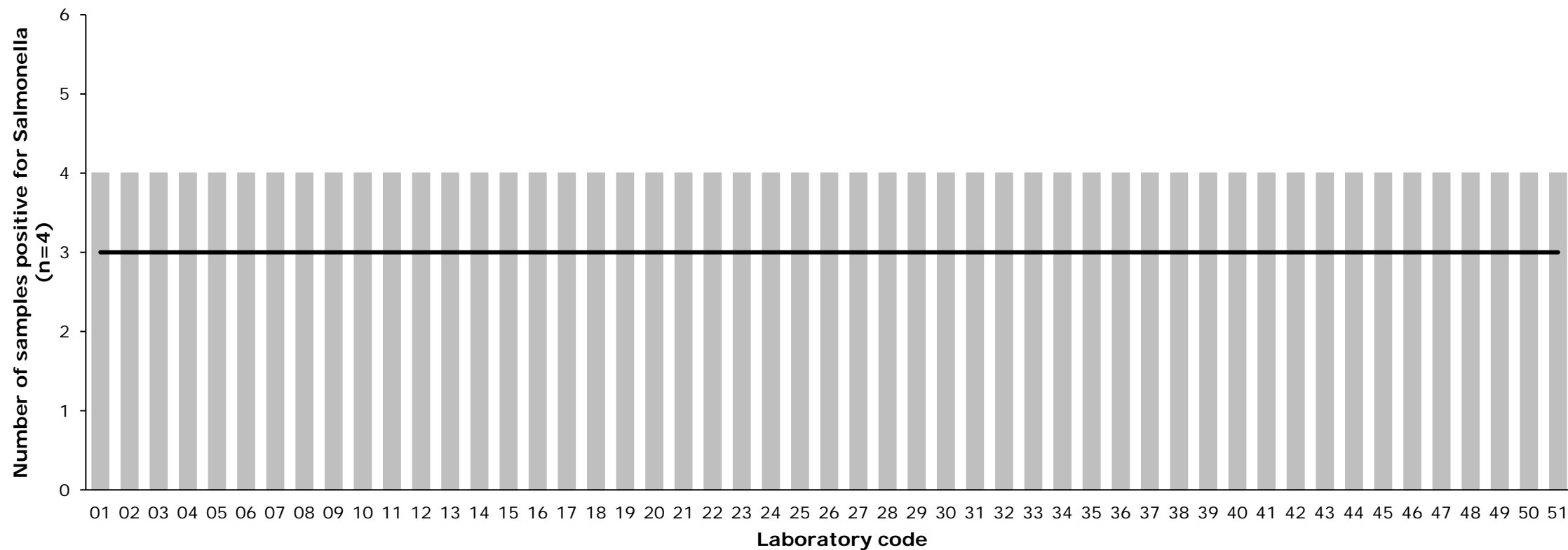
Criteria for good performance

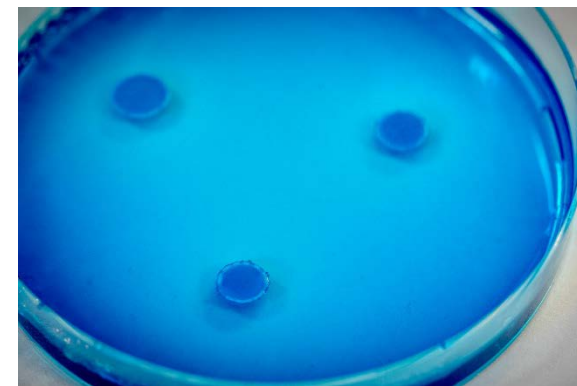
Artificially contaminated samples	Percentage positive	# pos samples / total # samples
High level of <i>S. Typhimurium</i>	≥ 75%	≥ 3 / 4
Low level of <i>S. Typhimurium</i>	≥ 50%	≥ 3 / 6
Negative samples	0%*	0 / 4*
Control samples	Percentage positive	# pos samples / total # samples
Procedure control (BPW only)	0%	0 / 1
Positive control with <i>Salmonella</i>	100%	1 / 1

* 100% *Salmonella*-free matrix cannot be guaranteed, so that an incidental positive result with a *Salmonella* strain different from the inoculation strain is still considered as acceptable.

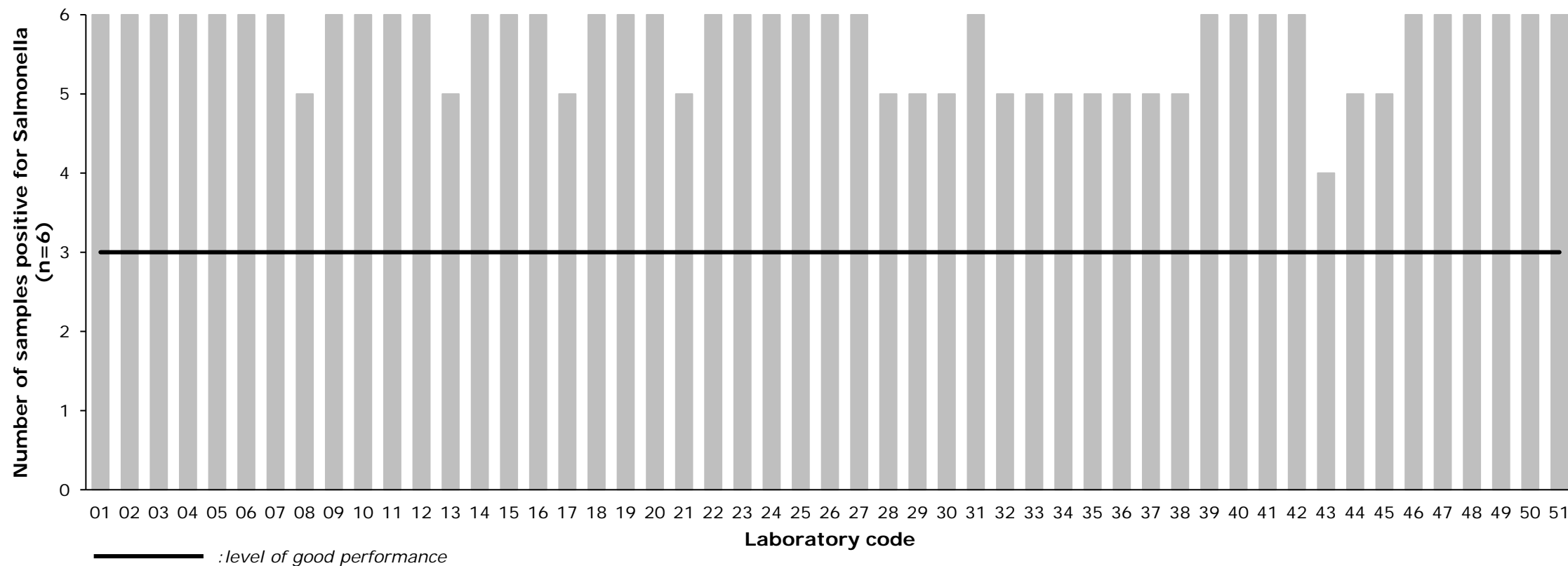


Results - High level contamination





Results - Low level contamination





Results

Flaxseed samples		All participants n = 51
High level STm (n = 4)	No. of samples	204
	No. of positive samples	204
	Sensitivity	100%
Low level STm (n = 6)	No. of samples	306
	No. of positive samples	288
	Sensitivity	94%
Negative (n = 4)	No. of samples	204
	No. of negative samples	204*
	Specificity	100%
All flaxseed samples artificially contaminated with <i>Salmonella</i>	No. of samples	510
	No. of positive samples	492
	Sensitivity	96%
All flaxseed samples	No. of samples	714
	No. of correct samples	696
	Accuracy	97%



Results

Control samples		All n = 51
Procedure control (BPW only) n=1	No. of samples	51
	No. of negative samples	51
	Correct score	100%
Positive control (own <i>Salmonella</i> control) n=1	No. of samples	51
	No. of positive samples	51
	Correct score	100%
All control samples n=2	No. of samples	102
	No. of correct samples	102
	Accuracy	100%



Performance

- **50 labs scored a good performance**
- 1 lab scored a moderate performance*
 - Made an administrative error during reporting in one of the negative samples.
 - * Waiting on raw data



Technical deviations

Laboratory code	BPW	MKTTn			RVS		MSRV		
	incubation (hours)	Concentration novobiocin (mg /L)	pH	Temperature (°C)	pH	Temperature (°C)	Concentration novobiocin (mg/L)	pH	Temperature (°C)
EN ISO 6579-1 (/A1:2020)	18 ± 2 hours	40 mg /L	7 - 8,2	34 °C - 38 °C	5,2 ± 0,2	41,5 °C ± 1 °C	10 mg / L	5,1 - 5,4	41,5 °C ± 1 °C
14	20	40	6,6	36	5,2	41,5			
19	18	40	8,2	41			10	5,3	41
20	20	20	8,02	37	5,48	41,5	10	5,43	41,5
21	20	20	8,02	37	5,48	41,5	10	5,43	41,5
24	18	40	6,8	41,5	5,2	37			
25	18							5,3	41,5
36	18	10	7,7	37	5,2	42	10	5,2	42
40	24	40	7,8	37	5,2	41,5			
41	19	20	8,05	37	5,0	41,5			
43	18	40	8,2	37			10	5,6	41,5
46	20	40	8,08	37	5,45	41,5	10	5,6	41,5
50	21 h 40 min.	39,02	7,3	37	5,3	41,5			
51	20	40	8	37	5,2	41,5	10	5,2	37



Positive control

<i>Salmonella</i> serovar	Number of participants
<i>S. Abaetetuba</i>	3
<i>S. Agbeni</i>	2
<i>S. Alachua</i>	1
<i>S. Blegdam</i>	2
<i>S. Enteritidis</i>	13
<i>S. Harleystreet</i>	2
<i>S. Infantis</i>	3
<i>S. Nottingham</i>	7
<i>S. Poona</i>	1
<i>S. Tranoroa</i> (<i>Salmonella enterica</i> subsp. <i>salamae</i>)	1
<i>S. Typhimurium</i>	15
<i>Salmonella bongori</i> serovar 66 : z41: -	1

Media capable of supporting the growth of the target organisms in low numbers?

Concentration <i>Salmonella</i> (cfu/sample)	Number of participants
1 - 10	14
11 - 20	6
21 - 120	18
250 - 500	4
$10^3 - 10^8$	7
Not Determined	2



Second detection method

- › Used (routinely) in laboratories to analyse samples
 - Not assessed for performance

- › 19 participants reported results of a second detection method
 - 15 NRLs-*Salmonella* used Real-Time PCR
 - 3 NRLs-*Salmonella* used VIDAS
 - 1 NRL-*Salmonella* used PCR

- › Similar results to the prescribed method EN ISO 6579-1:2017(/Amd 1:2020)



Next

- › Individual results were sent
- › Interim summary in the coming weeks
- › Report





Thank you!
Questions?



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