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

EURL- *Salmonella*

Proficiency test on the detection of *Salmonella* in hygiene swab samples (2020)

Irene Pol-Hofstad

EURL- Salmonella | 28 May 2021

EURL-*Salmonella* Detection study 2020
Primary Production Stage and Food

- Matrix: hygiene swabs
- Swab surfaces in food production areas and poultry farms

- Hygiene swabs contaminated at EURL
  - background flora
  - high and low concentration *Salmonella Typhimurium*

Preparation of samples

- Sterile hygiene swabs in whirlpool bags
- Moisturised with 10 ml peptone saline solution
- Add 1 ml of 10^8 cfu/ml background flora:

  Escherichia coli and *Citrobacter freundii*

- Add *Salmonella* Typhimurium
  - Low: approx. 5-10 cfu
  - High: approx. 50-100 cfu

Determination background flora (PT 2017)

- Which strains do not interfere with confirmation tests *Salmonella*

Media / Micro-organism	MSRV	*BSA*	XLD	BGA
***S.* Typhimurium**	**Clear Hallow**	**Black/green col.**	**Black (pink) colonies**	**Pink col. (red hallow)**
E. coli	No growth	White col.	Yellow col.	Green col.
K. pneumonia	No growth	Blue col.	Yellow col.	Green col.
E. cloacea 1	No growth	Blue/green col.	Yellow col.	Green col.
E. cloacea 2	No growth	Light green col.	Yellow col.	Green col.
P. aeruginosa	No growth	Pink col.	Small pink colonies	Pink col.
C. freundii	No growth	White col.	Yellow col.	Green col.

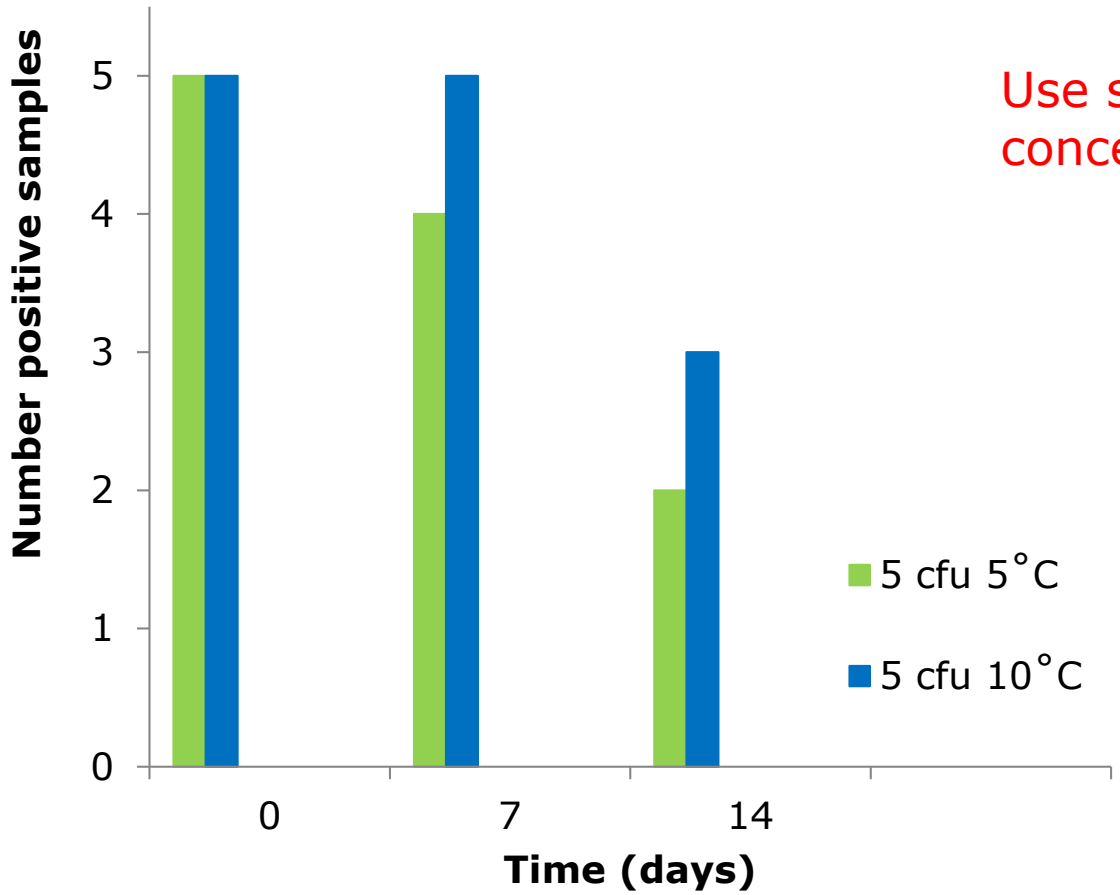
Pre-test: Stability of samples

- Simulate transport and storage conditions

- Storage conditions: 5 °C and 10 °C
  for up to 3 weeks

- Test for:
  - *Salmonella:* EN-ISO 6579:1-2017
  - *Enterobacteriaceae:* EN-ISO 21528-2

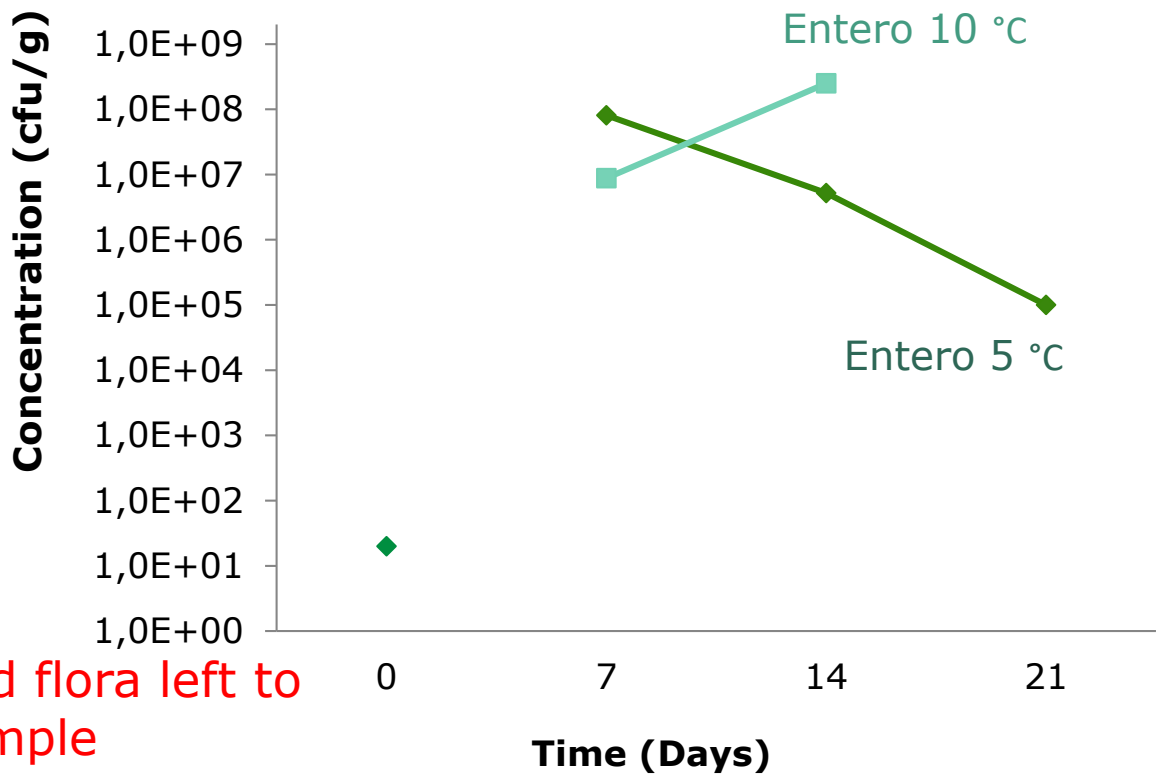
Results Pre-tests: *Salmonella* Typhimurium



Use slightly higer concentration *Salmonella*

Results Pre-tests: background flora

– *Enterobacteriaceae*



Enough background flora left to mimick real life sample

Proficiency Test design

- Matrix: Hygiene swabs
- Moisturised with 10 ml peptone saline solution
- Contaminated with 1 ml 10^8 cfu/ml background flora
- Contaminated with *Salmonella* Typhimurium ATCC 14028:
  - 6 x Low level STM: 8 cfu
  - 4 x High level STM: 50 cfu
  - 4 x Negative samples (No *Salmonella* added)

- Process controls:
  - C1: Hygiene swab + 10 ml PSS
  - C2: Hygiene swab + 10 ml PSS
    + own positive control

Participants

In total 67 participating NRLs

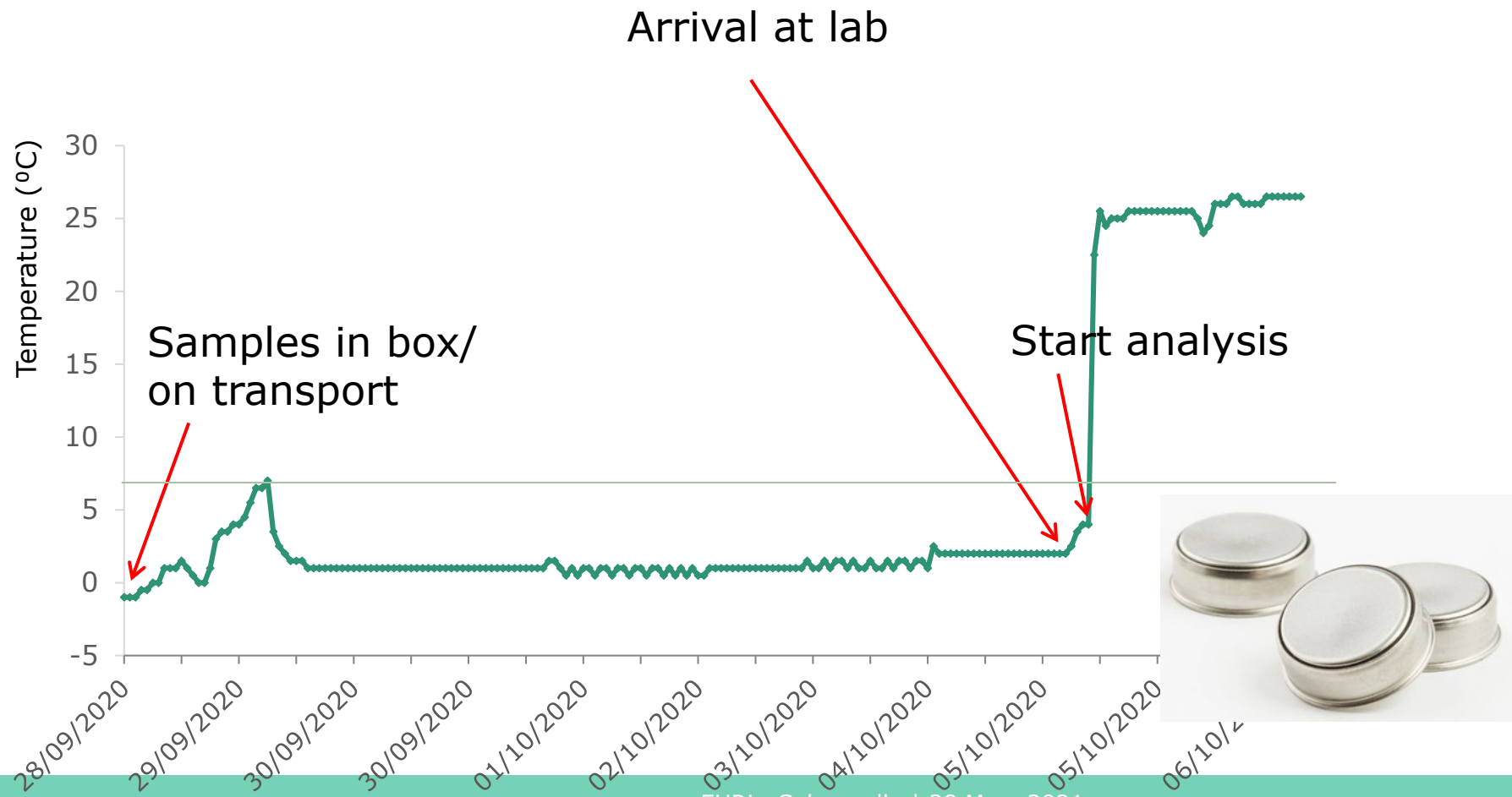
- 37 NRLs PPS and 30 NRLs Food
- from 28 EU Member States
- 5 NRLs from EU (potential) candidate Member States or European Free Trade Associations (EFTA)
- 1 NRL from a third country (on request of DG-Sante)

Transport

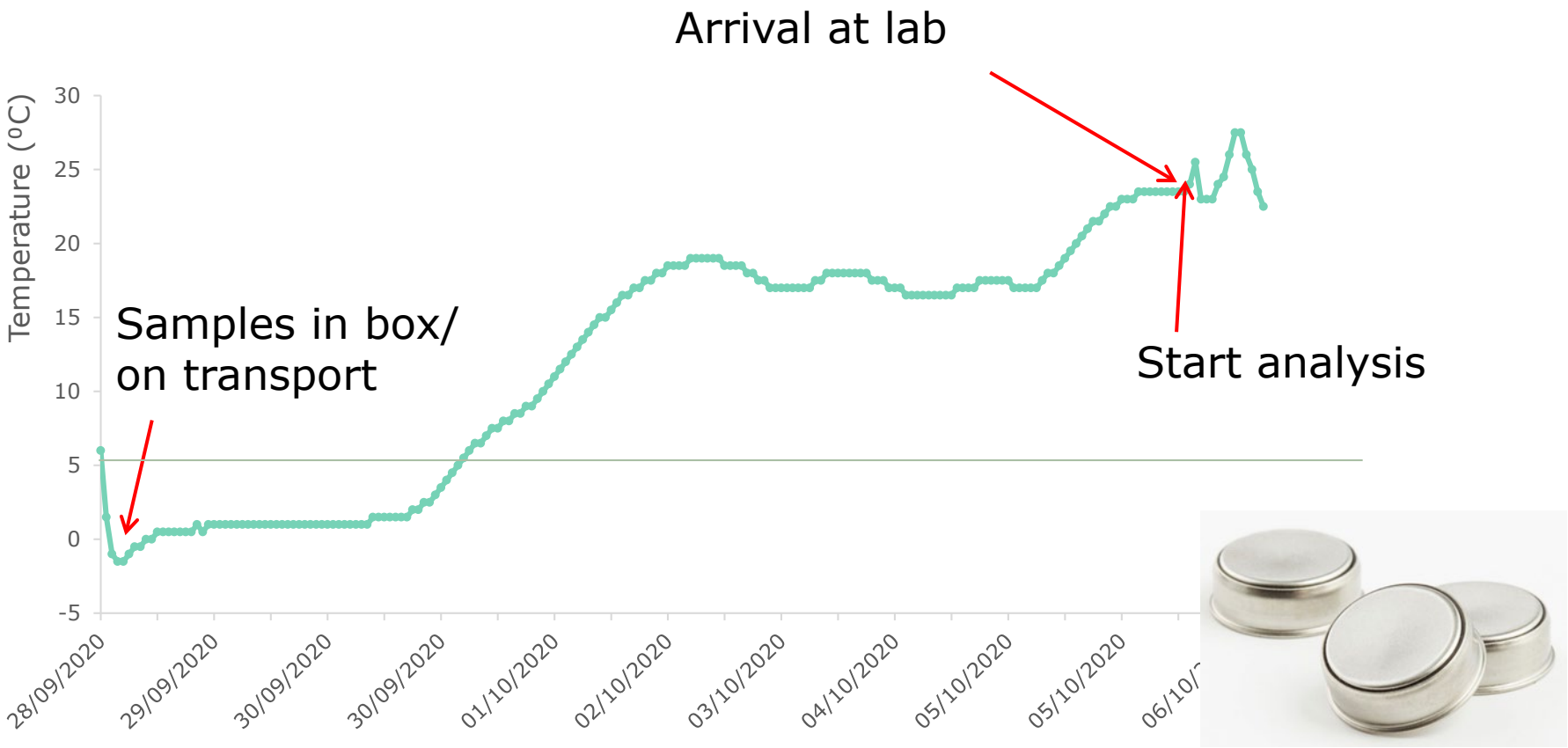
- Transport time:
  - 44 parcels: 0-1 day
  - 10 parcels: 2 days
  - 5 parcels: 3 days
  - 3 parcels: 4 days
  - 3 parcels: delays at the border (arrived after 8, 9 or 17 days)

- Temperature during transport and storage:
  - temperature probe in between samples
  - transport temperature: predominantly - 4°C and + 7 °C
  - storage temperature: mostly between 0 °C and 10 °C

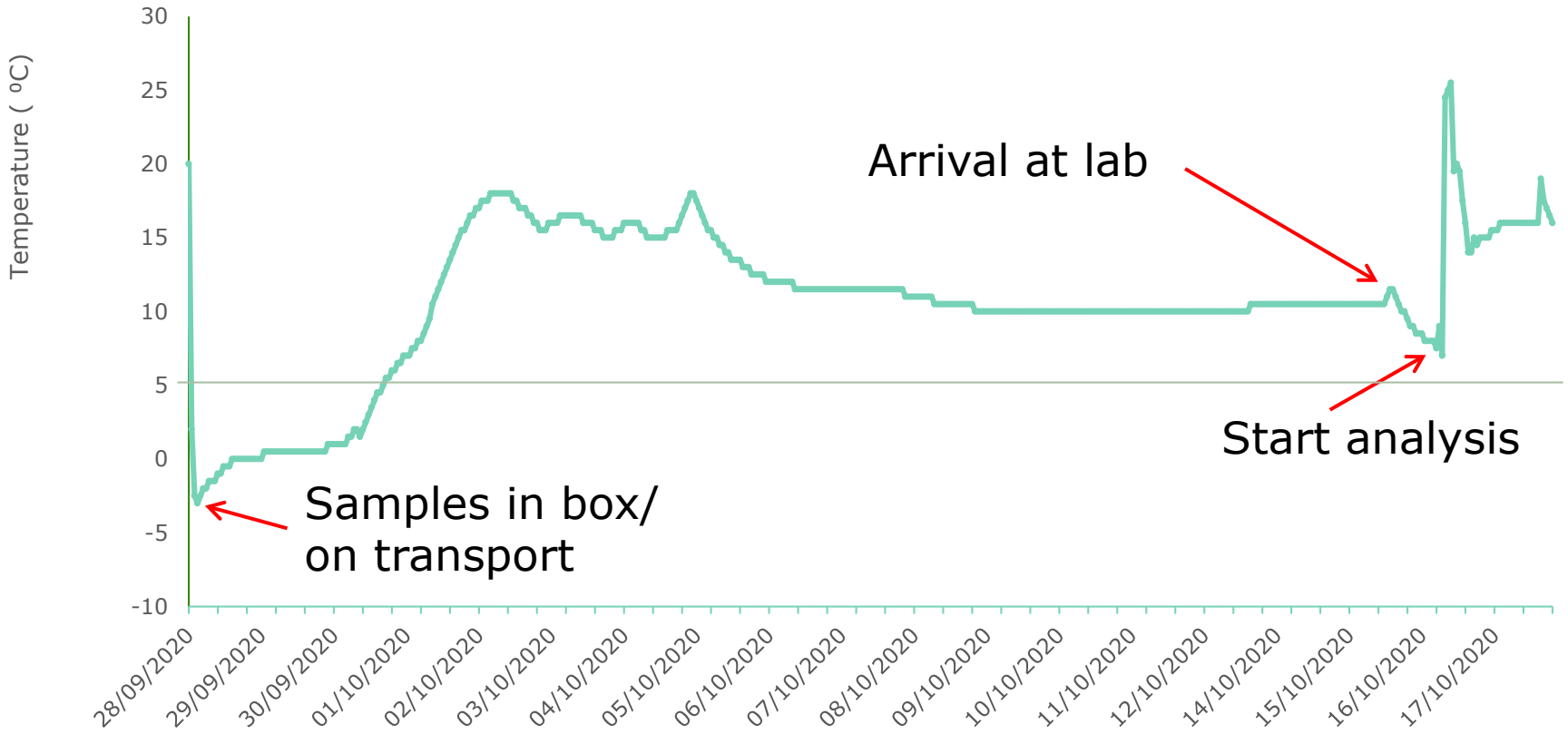
Temperature samples parcel Lab 66 (8 days)



Temperature samples parcel Lab 30 (9 days)



Temperature samples parcel Lab 54 (17 days)



Method PT - PPS-Food 2020

Prescribed: EN ISO 6579-1:2017

- NRL PPS: Selective enrichment on MSRV
- NRL Food: Selective enrichment on MKTTn and RVS or MSRV
- Selective isolation medium: XLD & 2nd agar of choice
- Confirmation
- Second detection method

PT samples: Artificial contamination

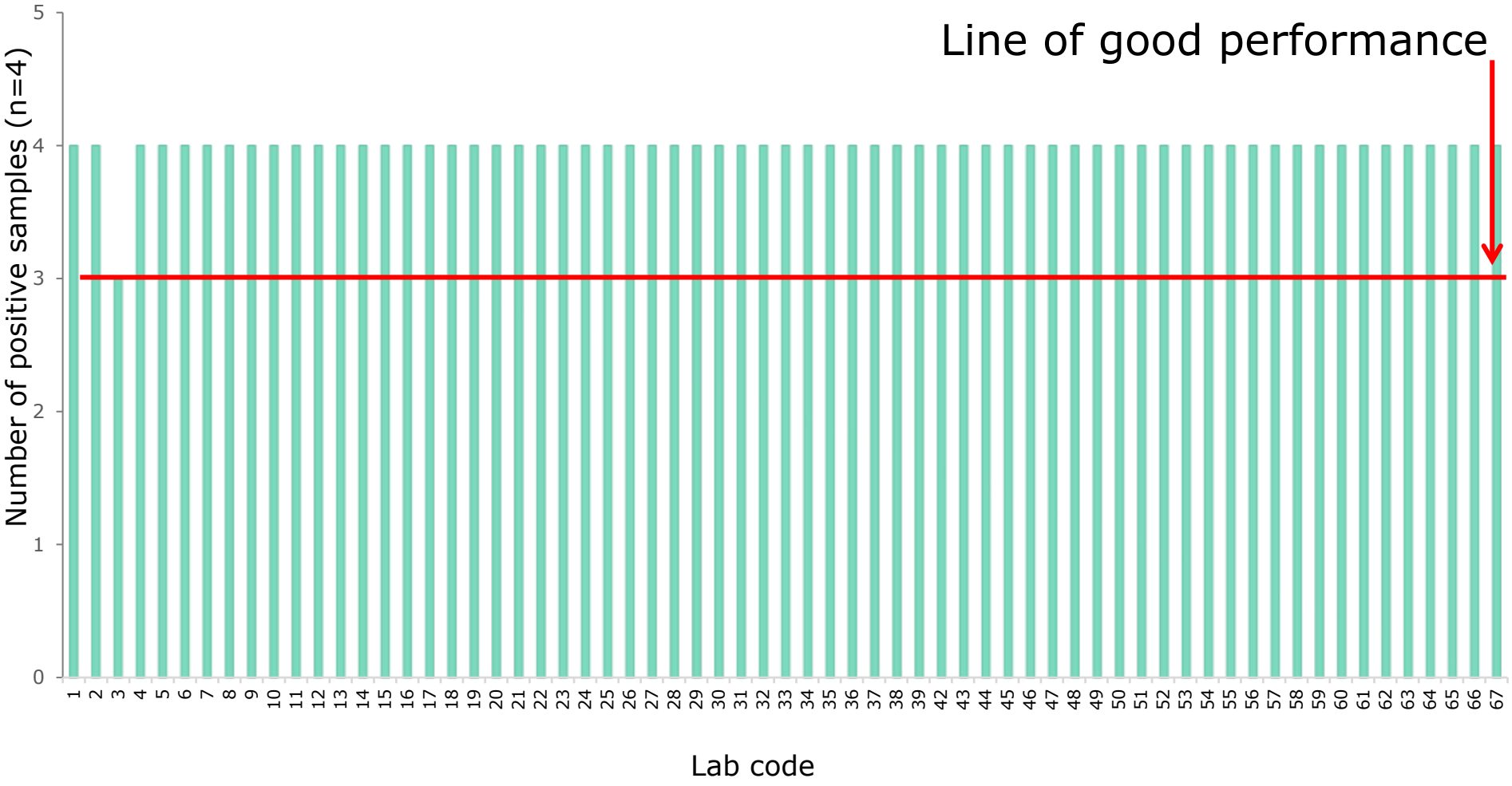
Date of testing	Low level STm cfu/sample	High level STm cfu/sample	Entero cfu/sample
23 Sept 2020 (Inoculum level of diluted culture)	7	47	$1{,}0 \times 10^8$
5 Oct 2020 MPN of inoculated hygiene swab samples (95% confidence limit)	3,3 (1,1 – 10,4)	35 (11 – 110)	$1{,}2 \times 10^8$

Results of participating laboratories

Criteria for good performance

Contamination level	% positive	# positive samples/ total # samples
Hygiene swab samples		
S. Typhimurium high-level	Min. 80 %	Min. 3/4
S. Typhimurium low-level	Min. 50 %	Min. 3/6
Negative (no *Salmonella* added)	0 %	0/4
Control samples		
Procedure control (BPW only)	0 %	0 /1
Positive control with *Salmonella*	100 %	1 /1

Results high concentration STm



Results: Low concentration STm

Samples: specificity, sensitivity, accuracy

Hygiene swab samples		All labs n = 65	EU NRL PPS n = 30	EU NRL Food n = 28
Negative samples n=4	No. of samples No. of negative samples Specificity in %	260 260 100%	120 120 100%	104 104 100%
Low level (STm) n=6	No. of samples No. of positive samples Sensitivity in %	390 384 98,5%	180 178 98,9%	156 153 98,1%
High level (STm) n=4	No. of samples No. of positive samples Sensitivity in %	260 259 99,6%	120 119 99,2%	104 104 100%
All Hygiene swab samples with STm	No. of samples No. of positive samples Sensitivity in %	650 643 98,9%	300 297 99,0%	260 257 98,8%
All Hygiene swab samples (positive and negative)	No. of samples No. of correct samples Accuracy in %	910 903 99,2%	420 417 99,3%	364 361 99,2%

Control samples: specificity, sensitivity, accuracy

Lab code 63 made administrative error in reporting their positive control as negative. Moderate performance

Lab code 66 misunderstood the purpose of the positive control sample and prepared their own positive control sample. Moderate performance

Control samples		All labs n= 65	EU NRLs n= 30	EU NRLs Food n = 26
Procedure control n = 1	No. of samples	65	30	26
	No. of negative samples	65	30	26
	Specificity in %	100%	100%	100%
Positive control (Own *Salmonella*) n= 1	No. of samples	65	30	26
	No. of positive samples	65	30	26
	Sensitivity in %	100%	100%	100%
All control samples n = 2	No. of samples	130	60	60
	No. of correct samples	130	60	60
	Accuracy in %	100%	100%	100%

Conclusions PT-PPS-Food 2020

- Control samples:
  - Excellent performance: 100% correct scores
  - Two labs: moderate performance
    - Administrative error: reporting positive control as negative
    - Misunderstanding purpose of sample C2

- Hygiene swab samples with *Salmonella* :
  - Negative samples: all labs scored all samples negative
  - High level STm: 1 lab scored 1 sample negative
  - Low level STm: 3 labs scored 1 sample negative and 1 lab scored 3 samples negative

63 labs scored good performance
2 labs scored moderate performance

Deviations from prescribed method

Lab code	BPW		RVS	MKTTn			MSRV		
	Incubation time	T (°C)	pH	T (°C)	pH	Novo-biocin	T (°C)	pH	Novo-biocin
EN ISO 6579-1	16–20 h	37	5.0–5.4	37	7.0–8.2	40 mg/l	41,5	5.1–5.4	10 mg/l
4	18:00	37	5,4	37	6,8	40 mg/l			
12	20:00	37					41,5	5,6	10 mg/L
13	20:00	37		37	7,8	40 mg/L	41,5	5,6	10 mg/L
17	22:00	37	5,31	37,2	7,16	40 mg/L			
18	19:00	37		41,5	8,1	40 mg/L	41,5	5,3	10 mg/L
21	18:00	37		37	8	10 mg/L	42	5,2	10 mg/L
27	19:00	37,1	5,3	41,5	7,8	0 mg/L			
33	20:00	36,9					41,5	5,3	20mg/l
38	18:00	36	5,3	36	6,8	mg/L			
39	18:00	37	5,2	37	8	40 mg/L	41,5	5,2	50 mg/L
42	18:00	37	5,46	37	8,01	10mg/L	41,5	5,43	20mg/L
45	19:00	37	5,1	37	8,1	10mg/L	41,5	5,2	20mg/L
46	19:46	37,1		41,8	8,15	40 mg/L	41,8	5,34	10 mg/L
50	20:00	37					41,5	5,1	16mg/L
51	20:00	37		37	8,1	40mg/L	41,5	5,1	16mg/L
54	20:00	37		37	8,61	40mg/L	41,5	5,54	10mg/L
63	24:00	37	5,2	37	8	mg/L 40			
66	18:00	37		37	7,55	10 mg/L	41,5	5,4	10 mg/L
67	18:00	37		37	8	40 mg/L	37	5,4	20mg/L

Second detection method PT-PPS-Food 2020

- 20 labs performed second detection method:
  - PCR methods

- All but two labs were validated for PCR

- 19 labs found identical result to prescribed method
  - 1 lab: 1 low level sample negative

Thank you all for your participation in this study !

Week	Date	Subject
27	Week of 5 July	E-mailing the link to the registration form for the detection study. Please register by 30 August at the latest.
38	Week of 20 September	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373).
38	Week of 20 September	E-mailing the link for the result form to the participants. E-mailing the protocol and instructions for the result form to the NRLs. Preparation of media by the NRLs.
39	Monday 27 September	Performance of the Proficiency Test.
43	29 October 2021 at the latest	Deadline for completing the result form: 29 October 2021 (23:59h CET) After this deadline the result form will be closed