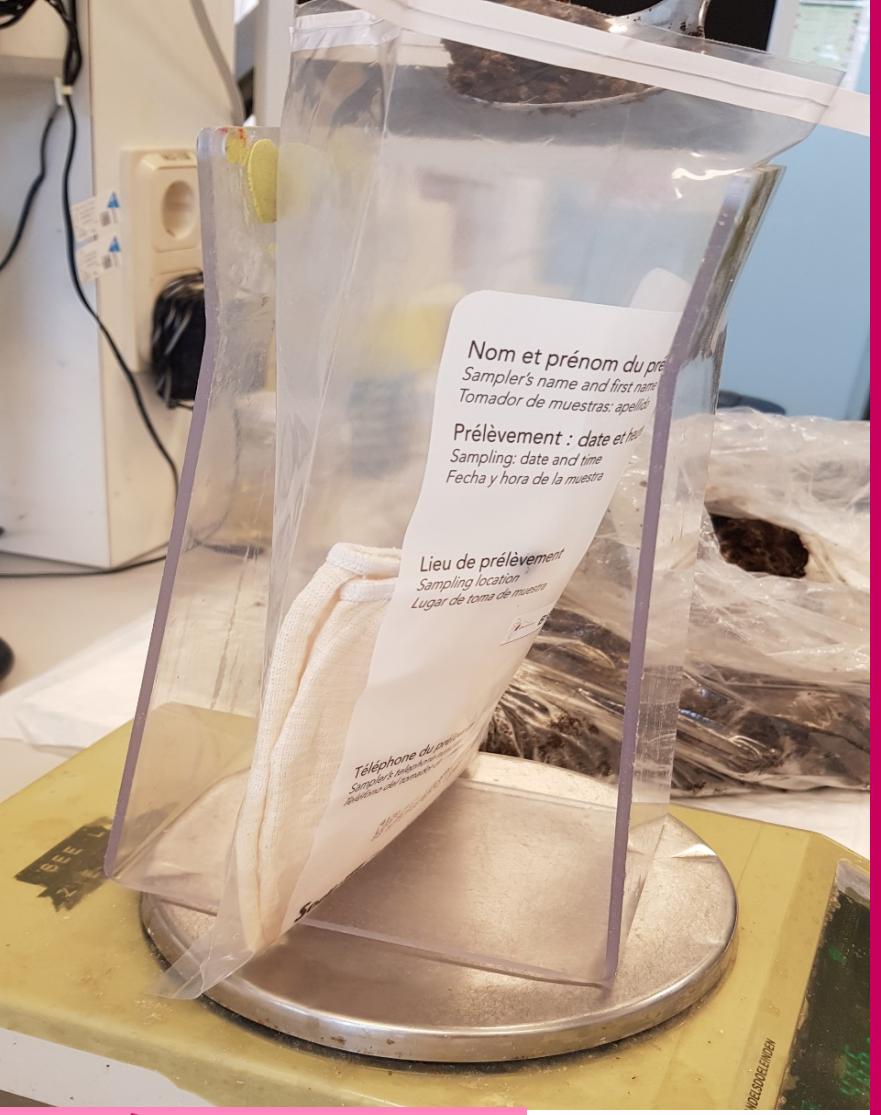


National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

EURL- *Salmonella*

Proficiency test on the detection of *Salmonella* in boot socks with chicken faeces (2021)

Irene Pol-Hofstad

EURL *Salmonella*

Co-funded by the European Union

EURL-*Salmonella* Detection study PPS 2021

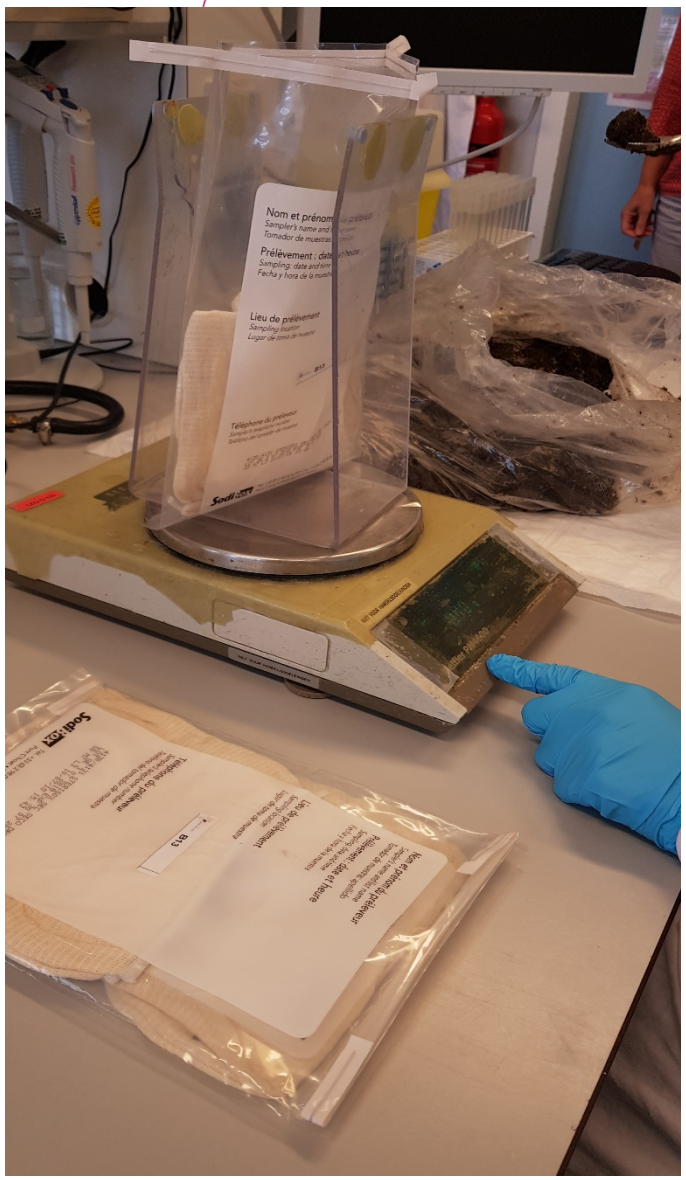
- Matrix: Boot socks with chicken faeces
- Boot socks are used to sample poultry farms
- Boot socks with chicken faeces contaminated at EURL
  - high and low concentration of *Salmonella* Infantis

Pictures: from Rapid microbiology.com and Gelderlander.nl

Preparation of samples

- 10 ml Peptone Saline Solution to moisten boot socks
- 15 g of chicken faeces

- Add high and low concentration *Salmonella* Infantis
  - Low: approx. 5-10 cfu
  - High: approx. 50-100 cfu



Pre-test: Stability of samples

- Simulate transport and storage conditions

- Pretest:
  - incubation contaminated boot sock samples at 5 °C and 10 °C for up to 3 weeks

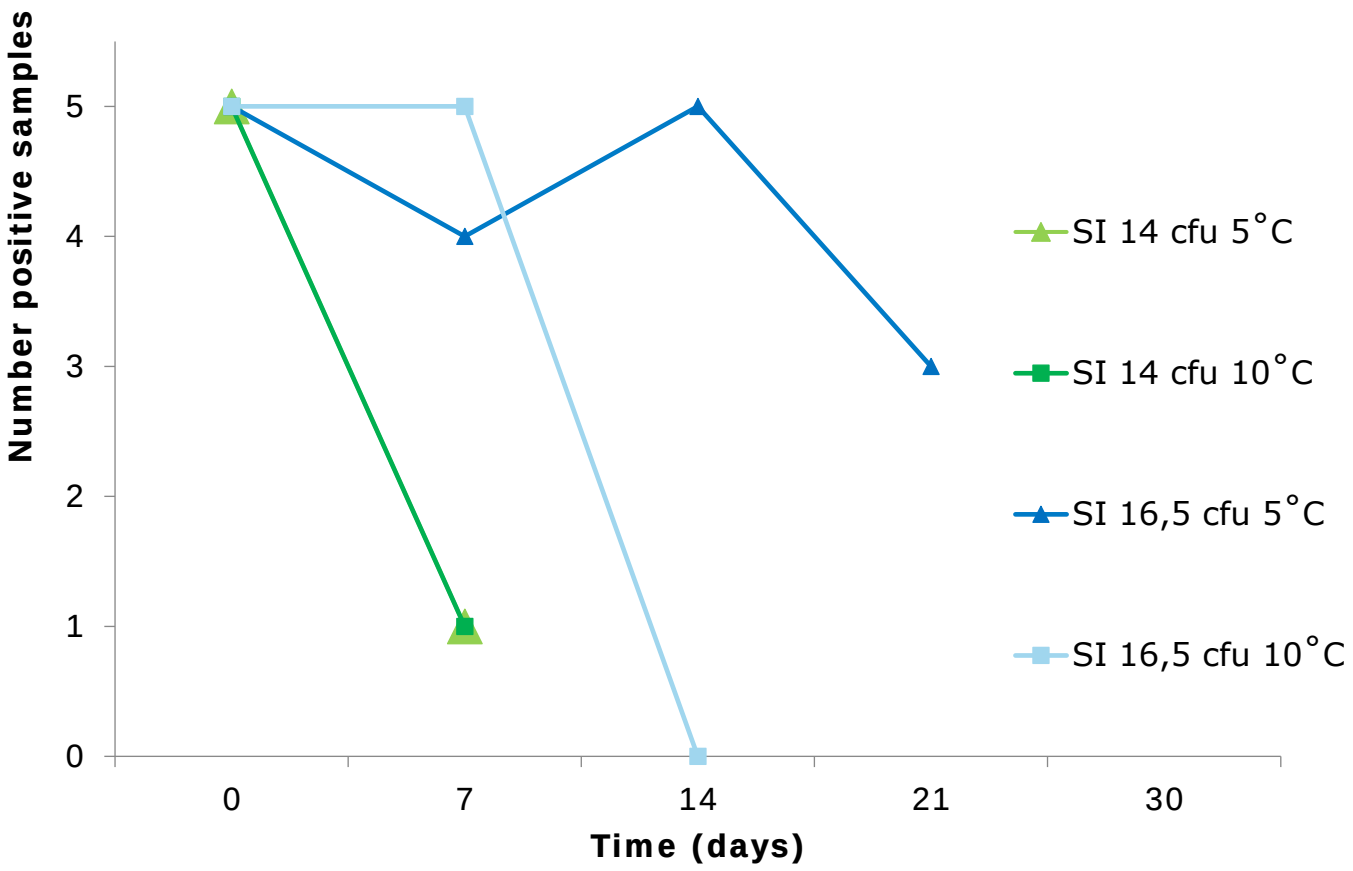
- Test for:
  - *Salmonella:* EN-ISO 6579:1-2017
  - *Enterobacteriaceae:* EN-ISO 21528-2
  - Aerobic count: EN-ISO 4833-1:2013

Conclusion Pre-tests: *Salmonella*

- Number of positive samples decreases over time

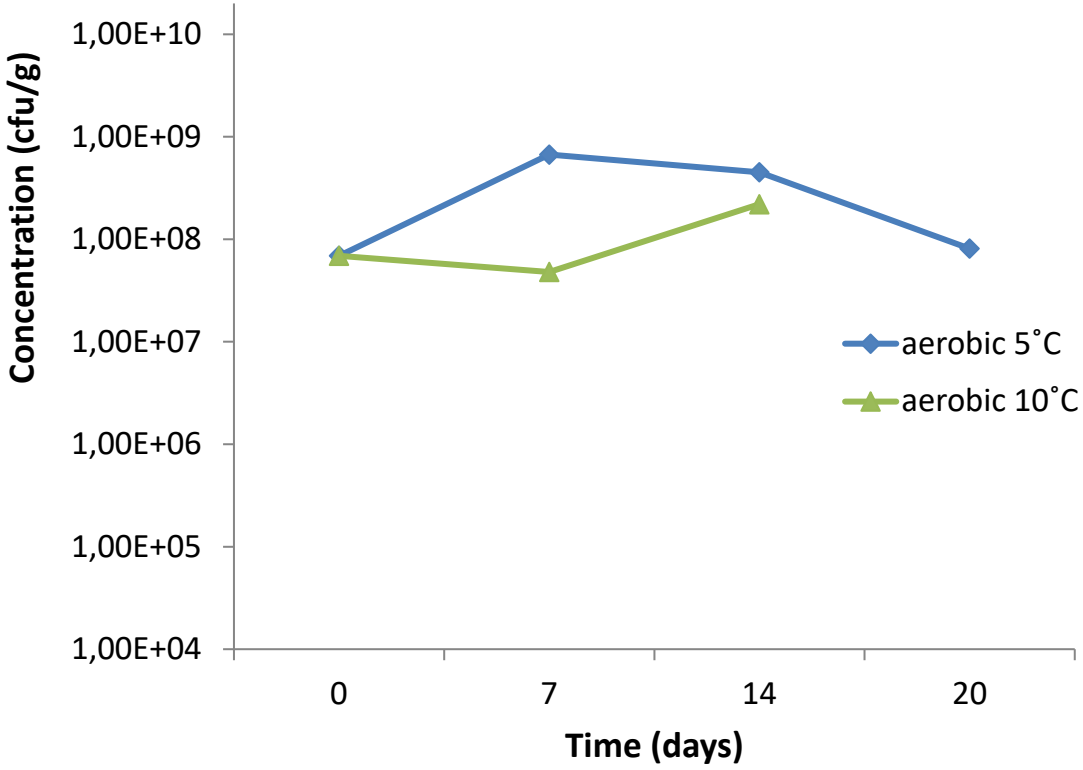
- Decreases faster with higher storage temperature

  > 16 cfu



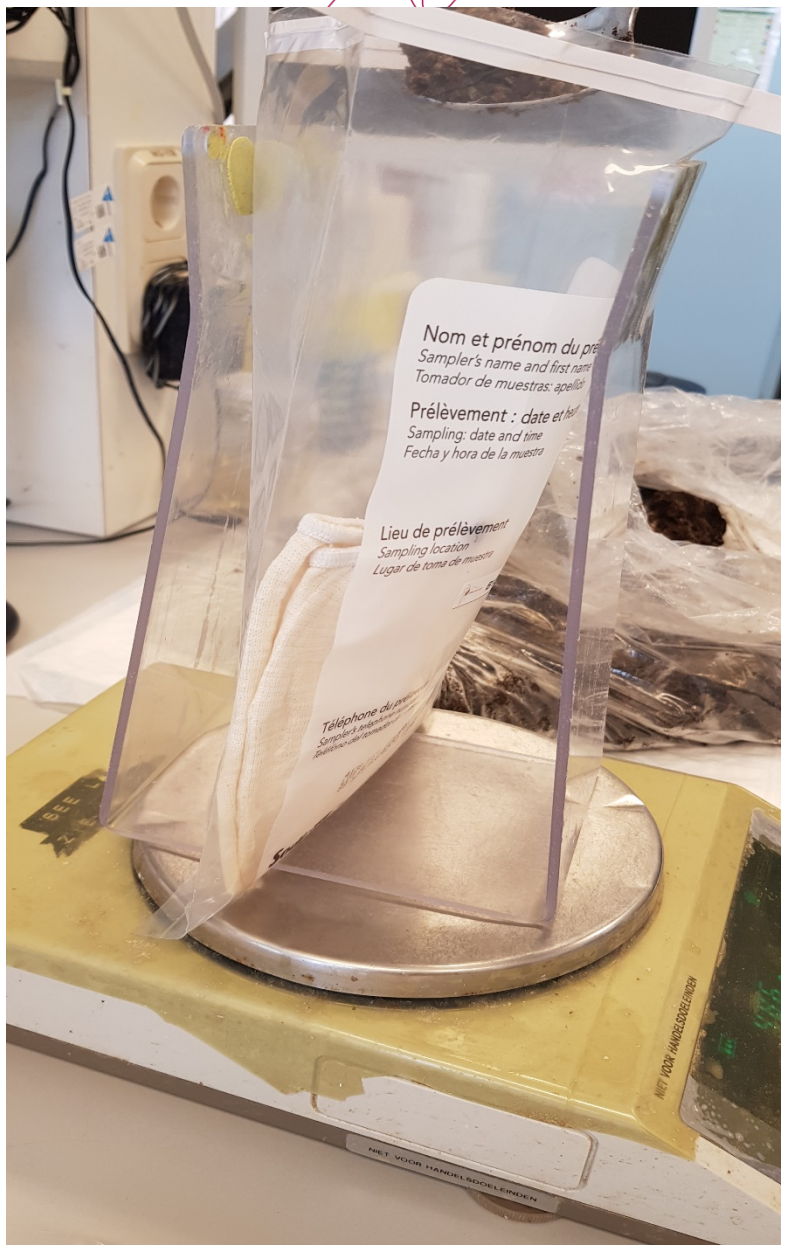
Conclusion Pre-tests: background flora

- *Aerobic count is stable*
- No *Enterobacteriaceae* in samples



Study design

- Boot sock + 10 ml PPS
- 15 g chicken faeces
- Contaminated with *Salmonella* Infantis:
  - 6 x Low level SI: 17 cfu
  - 4 x High level SI: 50 cfu
  - 4 x Negative samples (No *Salmonella* added)
- Process controls:
  - C1: Boot sock + 10 ml PSS
  - C2: Boot sock + 10 ml PSS
    + own positive control



Participants

In total 35 participating NRLs

- 27 NRLs from 27 EU Member States
- 6 NRLs from EU (potential) candidate Member States or European Free Trade Associations (EFTA)
- 1 NRL from a third country

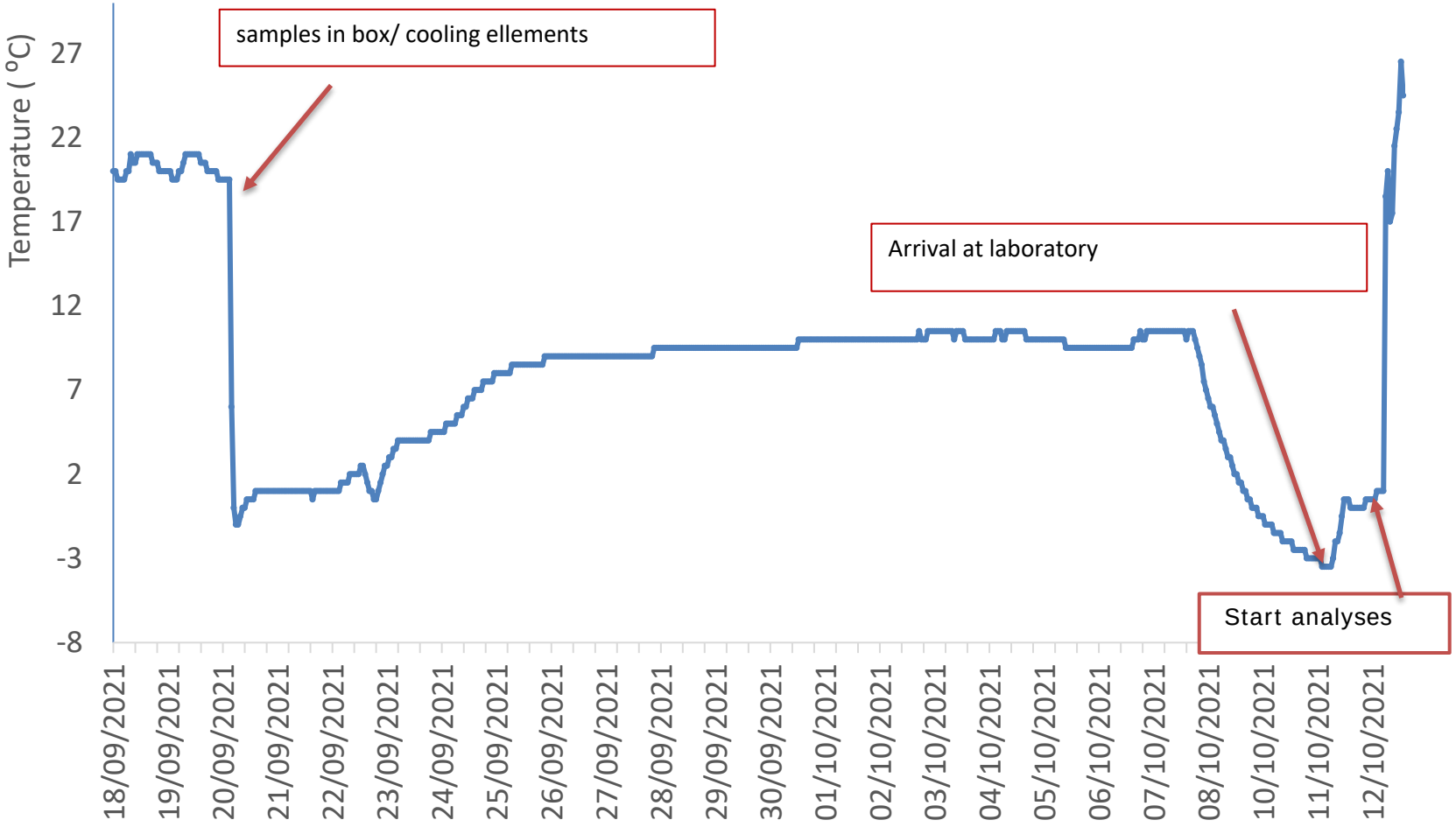
Pictures: bundeskanzler.de; Adam Smith Institute; De Veiligheidsdiplomaat (rijksoverheid.nl); emnnetherlands.nl

Transport

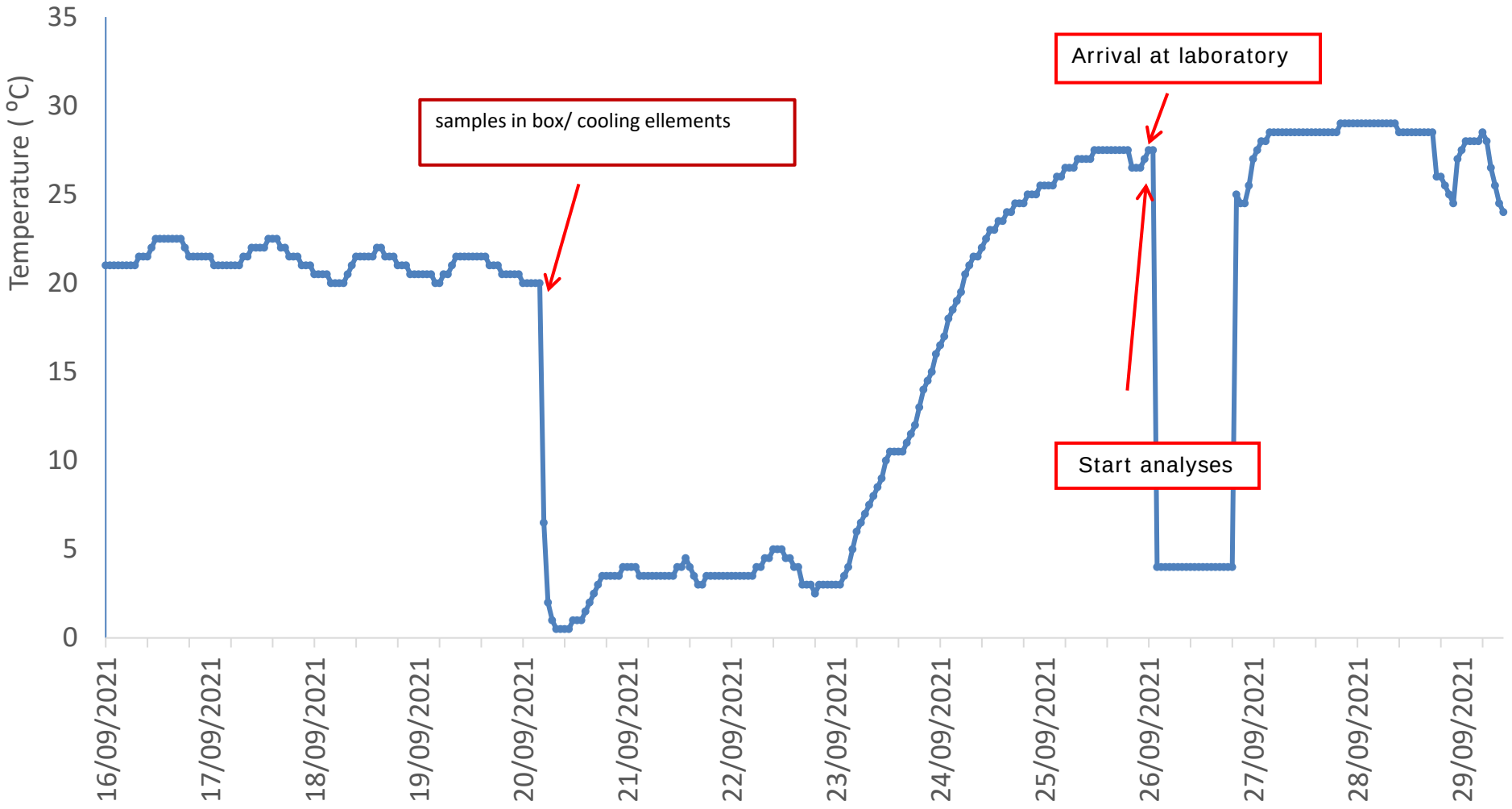
- Transport time
  - 17 parcels: 2 days
  - 11 parcels: 3 days
  - 2 parcels: 4 days
  - Lab code 22: 6 days
  - Lab code 10: 7 days
  - Lab code 30: 8 days
  - Lab code 27: 9 days
  - Lab code 21: 22 days (problems at customs)

- Temperature during transport and storage:
  - temperature probe between samples
  - transport temperature: predominantly -1,5 °C and + 7,5 °C
  - storage temperature: mostly 0 °C – 10 °C (labcode 8: at 17 °C !)

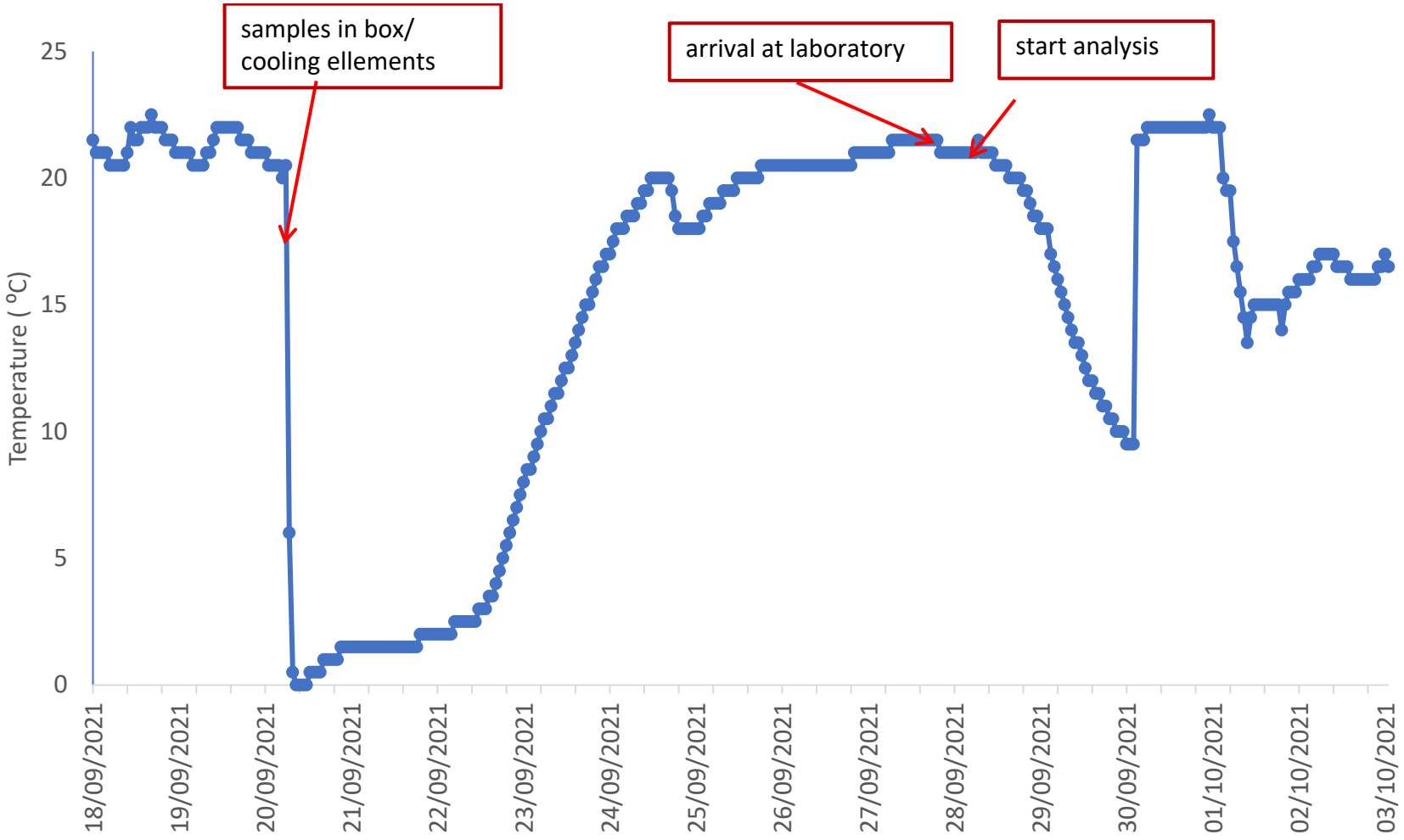
Temperature profile parcel 21



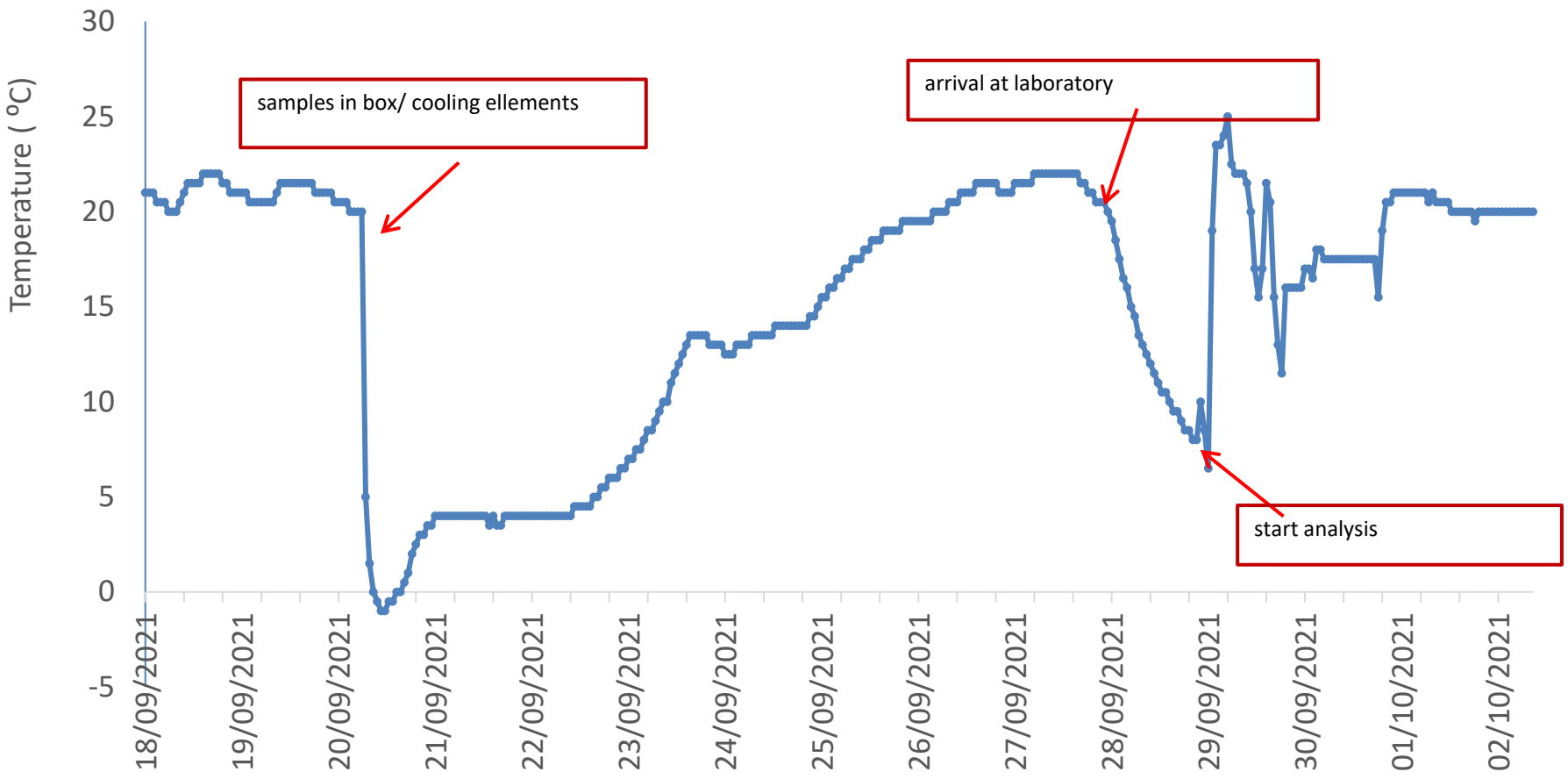
Temperature profile parcel 22



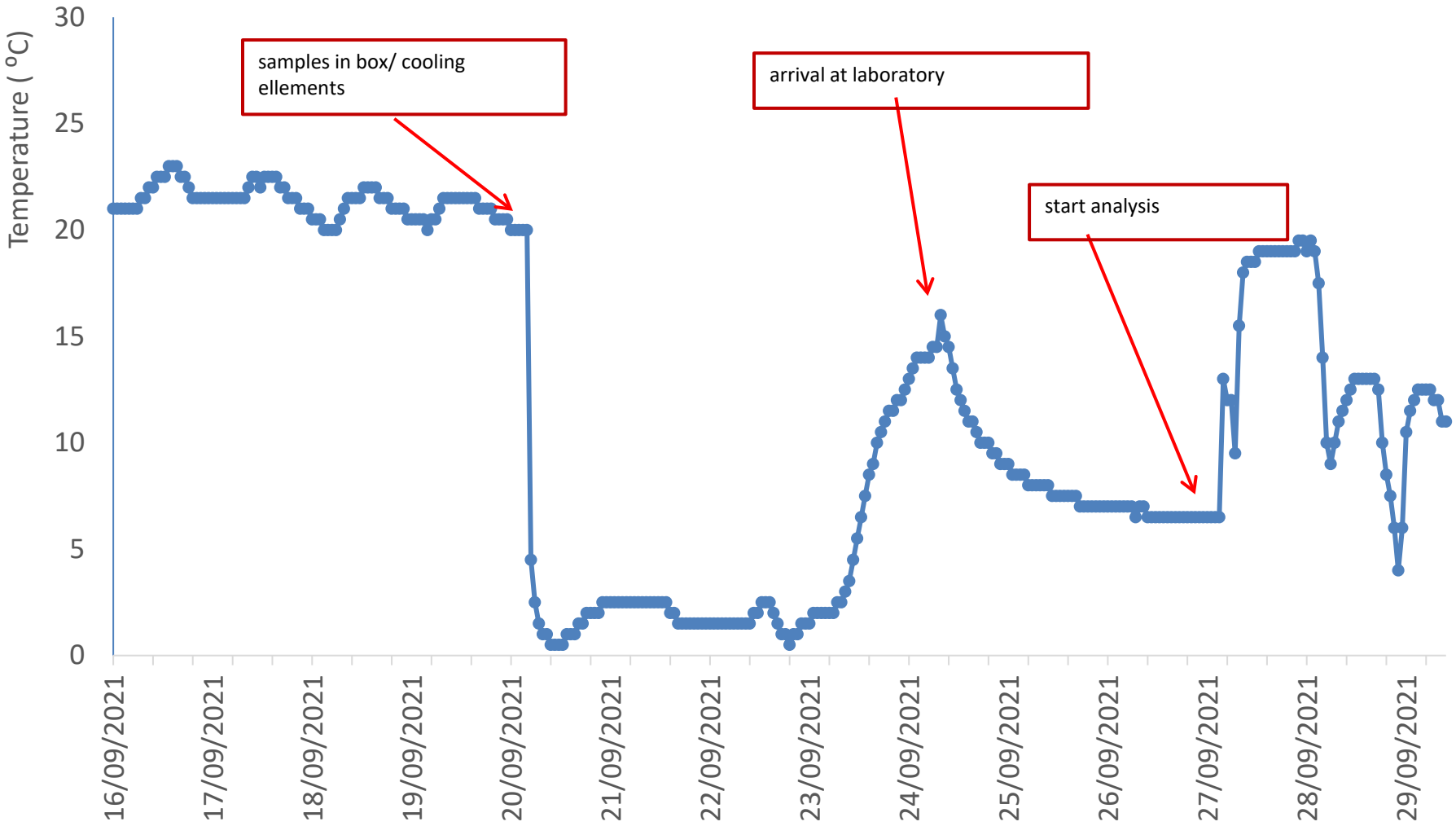
Temperature profile parcel 27



Temperature profile parcel 30



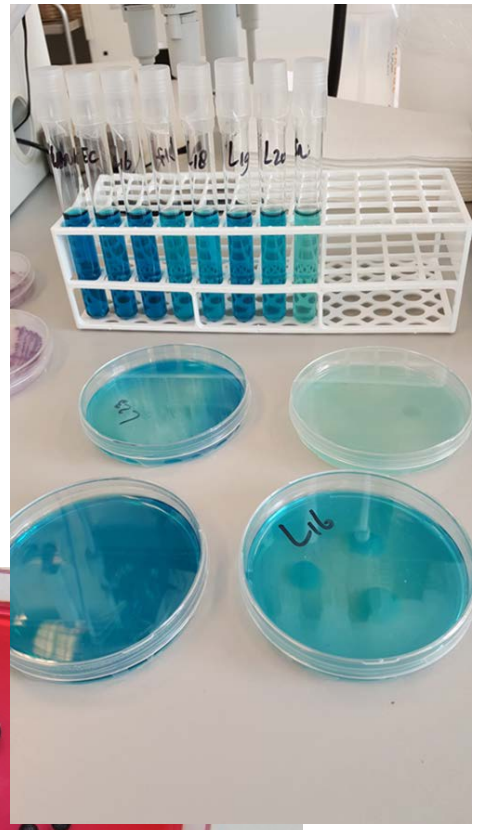
Temperature profile parcel 35

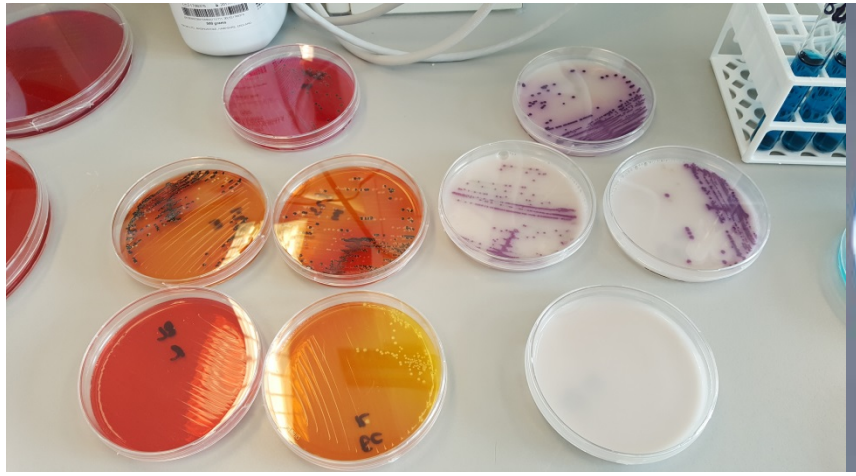


Method PT-PPS

Prescribed: ISO 6579-1:2017

- Selective enrichment on MSRV
- Isolation medium XLD & 2nd agar of choice





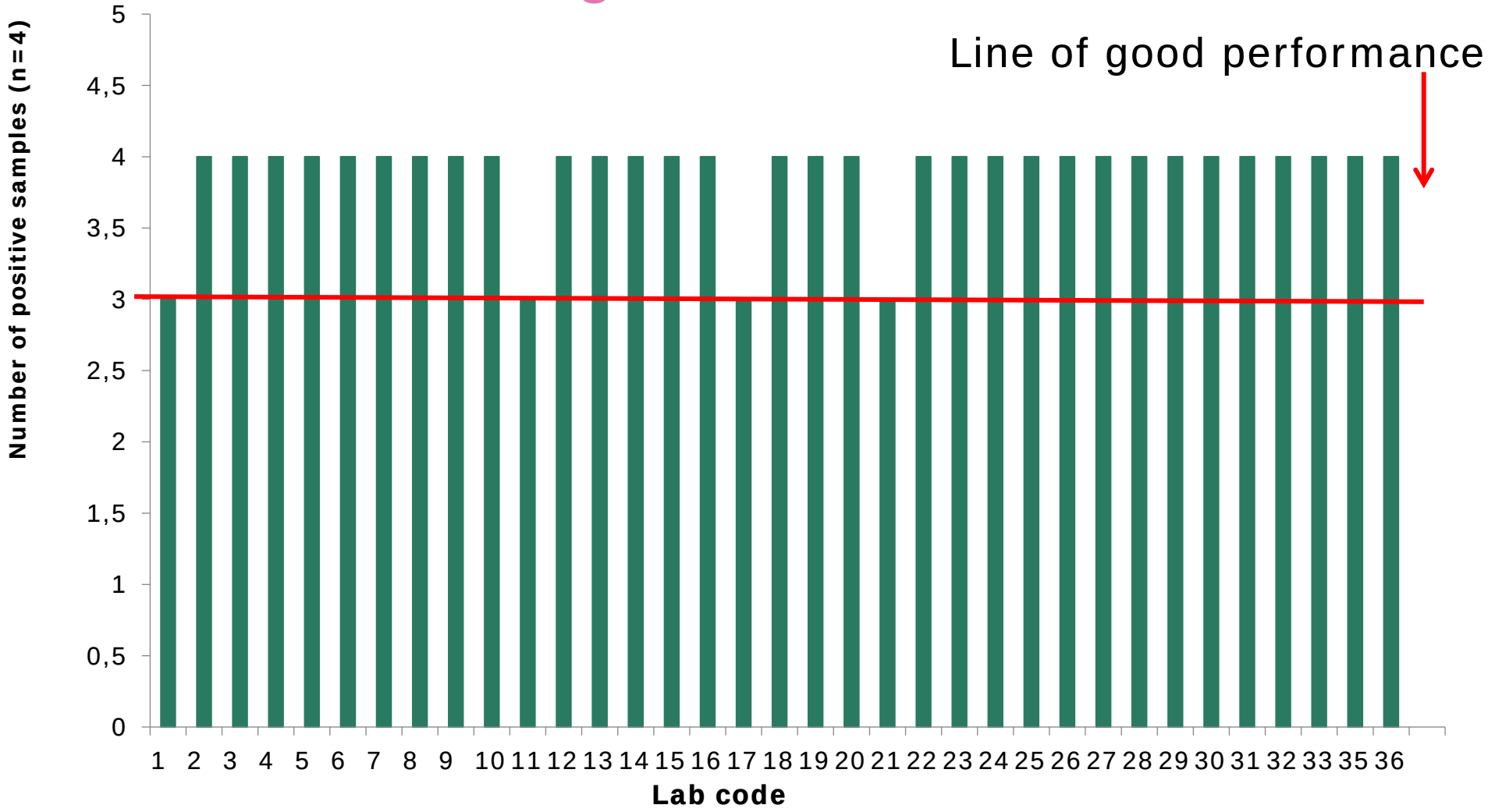
PT samples: Artificial contamination

Date of testing	Sample	Low level SI cfu / sample	High level SI cfu / sample
15 Sept 2021	Inoculum of diluted culture	12	31
27 Sept 2021	Inoculated boot socks with chicken faeces, stored 5°C MPN (95% confidence limit)	2,3 (0,78-7)	35 (11-110)
27 sept 2021	Inoculated boot socks with chicken faeces, stored 5°C	**Aerobic bacteria** $2,6 \times 10^8$	**Enterobacteriaceae** $1,0 \times 10^7$

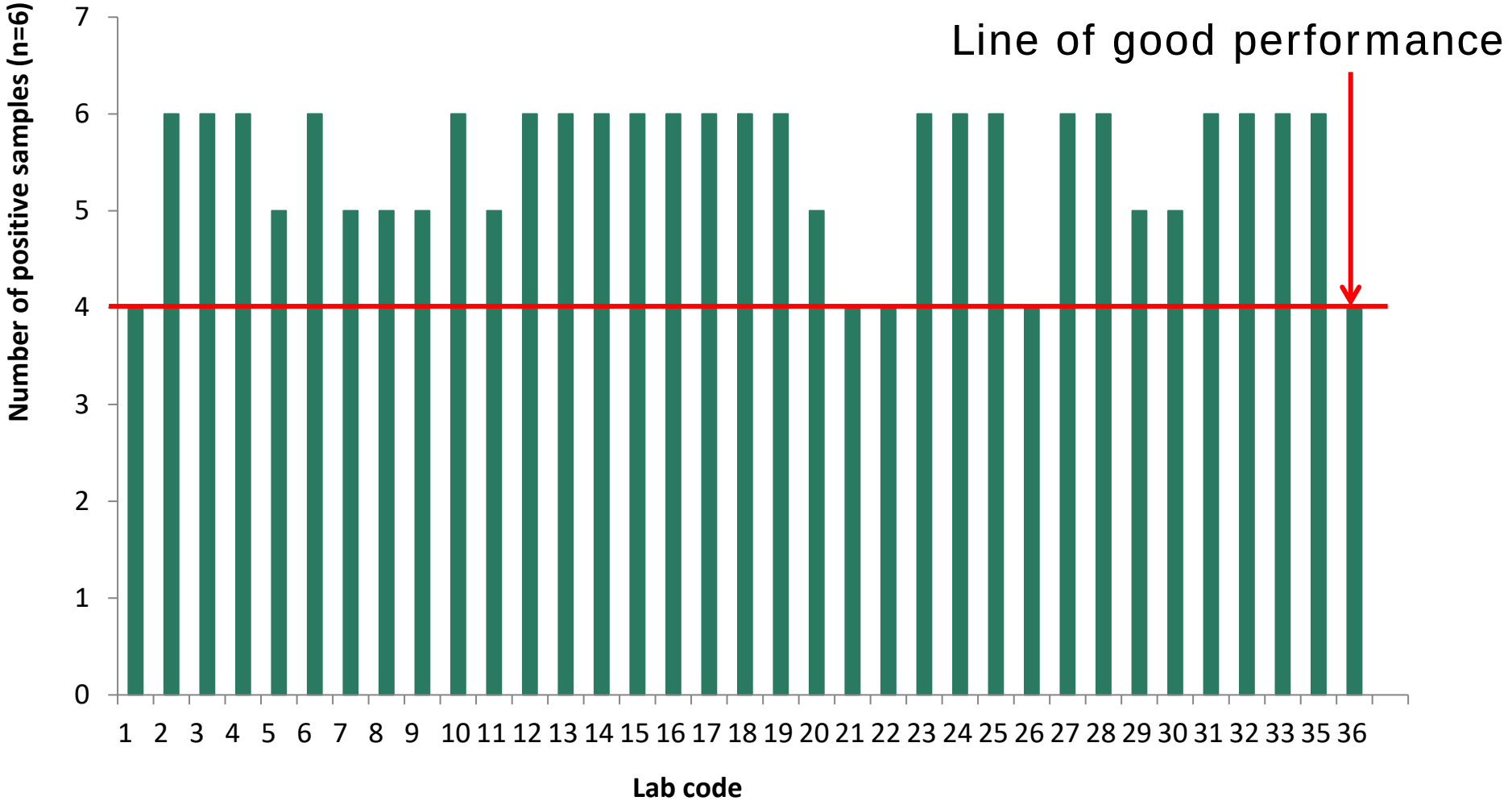
Deviations from ISO

Lab code	BPW		MSRV		
	Incubation time	T (ºC)	T (ºC)	pH	Novobiocin
EN ISO 6579-1	**16–20 h**	**37**	**41,5**	**5,1–5,4**	**10 mg/l**
21	**24**	37	42	5,1	-
27	18	37	41,5	**0**	**1000**
30	**18-24**	37	41,5	**RVS: pH 5,2**	**5 ml**

Results: High concentration SI



Results Low concentration SI



Samples: number of positive results

	Number of positive isolations		
	Negative n=4	SI low n=6	SI high n=4
Criteria good performance	0	≥3	≥3
Lab code 1 and 21	0	4	3
Lab code 11	0	5	3
Lab code 17	0	6	3
Lab code 22, 26 and 36	0	4	4
Lab code 5, 7, 8, 9, 20, 29 and 30	0	5	4
All other NRLs (n = 21)	0	6	4

Samples: specificity, sensitivity, accuracy

Boot sock samples		All participants n=35	EU NRLs PPS n=27
Negative samples n=4	No. of samples	140	108
	No. of negative samples	140	108
	Specificity in %	**100**	**100**
Low level SI n=6	No. of samples	210	162
	No. of positive samples	192	151
	Sensitivity in %	**91,4**	**93,2**
High level SI n=4	No. of samples	140	108
	No. of positive samples	136	105
	Sensitivity in %	**97.1**	**97.2**
All boot sock samples with SI n=10	No. of samples	350	270
	No. of positive samples	328	256
	Sensitivity in %	**93.7**	**94.8**
All boot sock samples (pos. and neg.) n=14	No. of samples	490	378
	No. of correct samples	468	364
	Accuracy in %	**95.5**	**96.3**

Control samples: specificity, sensitivity, accuracy

Control samples		All labs n = 35	EU NRLs PPS n = 27
Procedure control n=1	No. of samples	35	27
	No. of negative samples	35	27
	Specificity in %	**100%**	**100%**

Laboratory 18 made administrative error in reporting procedure control positive for *Salmonella* and their positive control as negative

Score: moderate performance

All control samples n=2	No. of samples	70	54
	No. of correct samples	70	54
	Accuracy in %	**100%**	**100%**

Conclusions PT-PPS 2021

- Control samples:
  - Excellent performance: 100% correct scores

- Boot sock samples with chicken faeces:
  - High level SI: 4 lab scored 1 sample negative
  - Low level SI: 8 labs scored 1 sample negative, and 5 labs scored 2 samples negative
  - Negative samples: all labs scored all samples negative
- 34 laboratories Good Performance, 1 laboratory Moderate Performance (lab code 18)

- Parcels experiencing temperature abuse scored good performance (1 or 2 low level samples neg)

- Parcel with long transport time scored 2 low and 1 high level samples neg

Thank you all for your participation in this study

EURL Salmonella

Timetable combined PT PPS-Food 2022

Week	Date	Subject
27-35		E-mailing the link to the registration form for the detection study. Please **register by 31 August 2022** at the latest.
39		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 26 September 2022	Shipment of the parcels to the participants as Biological Substance Category B (UN 3373).
40	Monday 3 October 2022	Performance of the Proficiency Test.
44	4 November 2022 at the latest	Deadline for completing the result form: **4 November 2022** (23:59h CET) After this deadline the result form will be closed.
	December 2022	Interim summary report