EURL- *Salmonella*

Proficiency test on the detection of *Salmonella* in boot socks with chicken faeces (2018)

Irene Pol-Hofstad

EURL- Salmonella | 29 May 2019

EURL-*Salmonella* Detection study PPS 2018

- 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

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

- 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
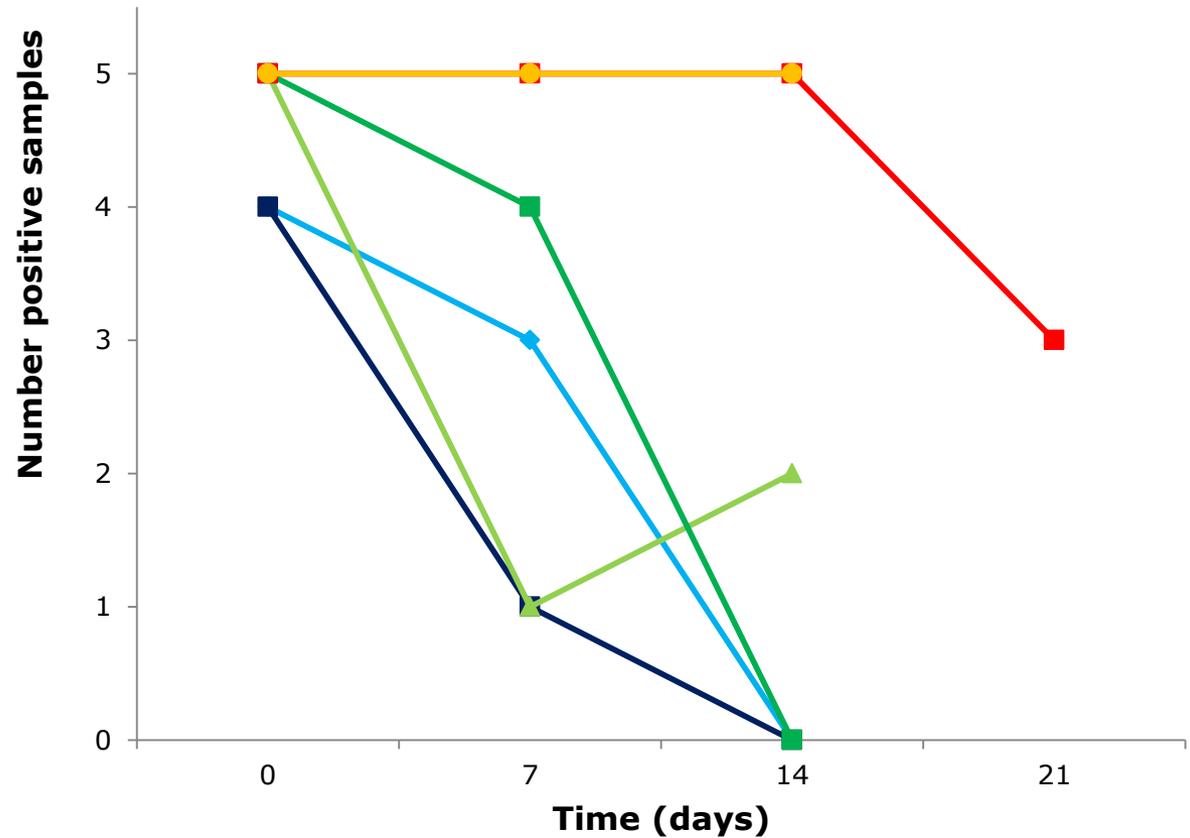 – Aerobic count: EN-ISO 4833-1:2013
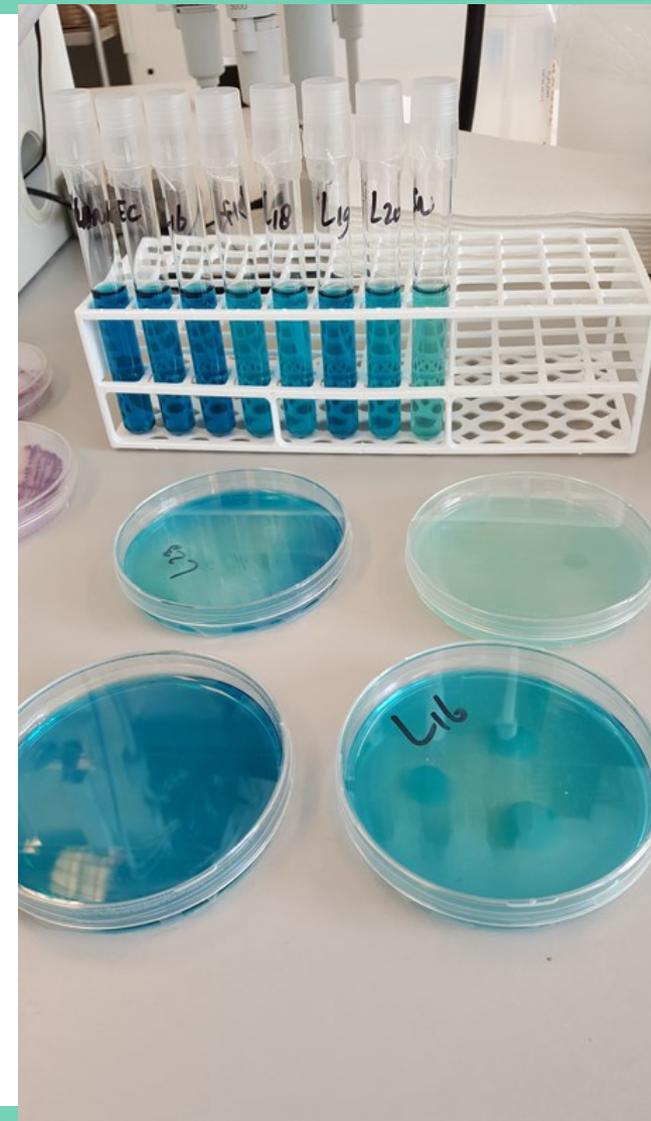
Conclusion Pre-tests: *Salmonella*
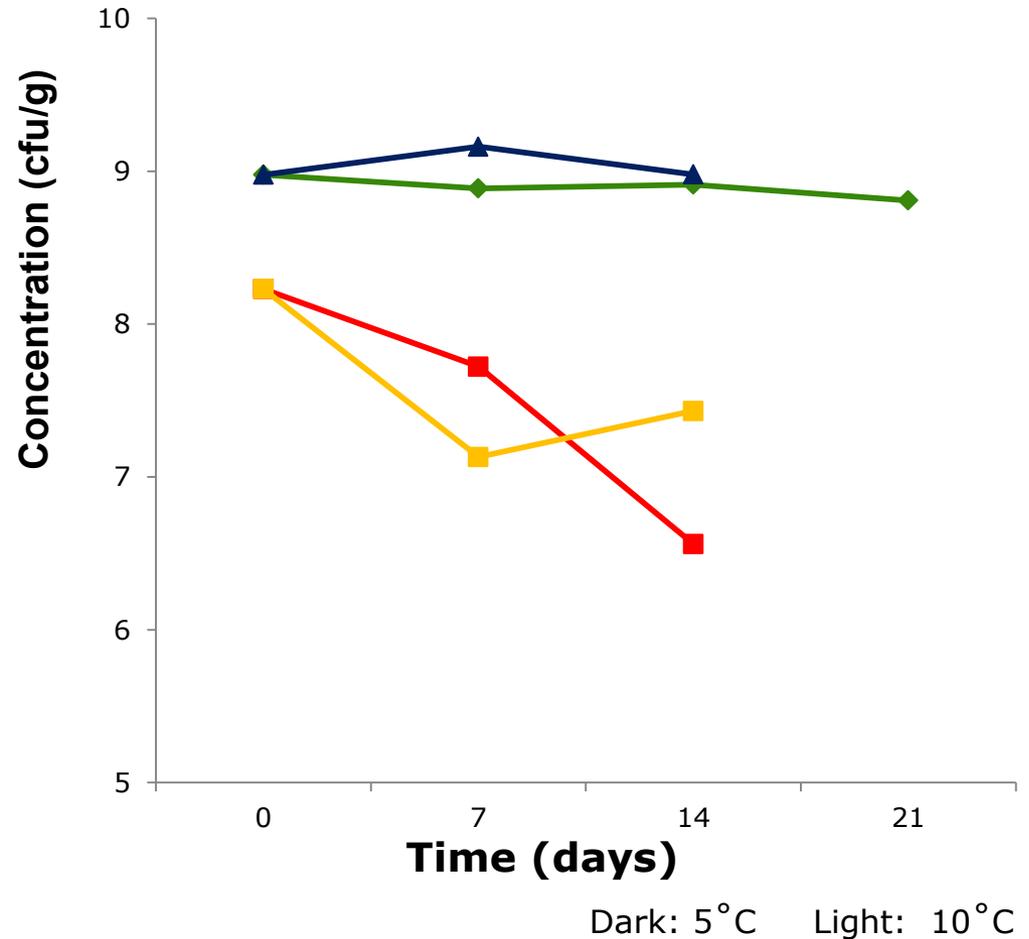
– *Salmonella*

  › # positive samples

  14 cfu

Dark: 5˚C   Light: 10˚C

Conclusion Pre-tests: background flora

– *Aerobic count*

– *Enterobacteriaceae*

Dark: 5˚C   Light: 10˚C

Study design

- Boot sock + 10 ml PPS

- 15 g chicken faeces

- Contaminated with *Salmonella* Infantis:
  – 6 x Low level SI: 14 cfu
  – 6 x High level SI: 50 cfu
  – 6 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 36 participating NRLs

- – 29 NRLs from 28 EU Member States

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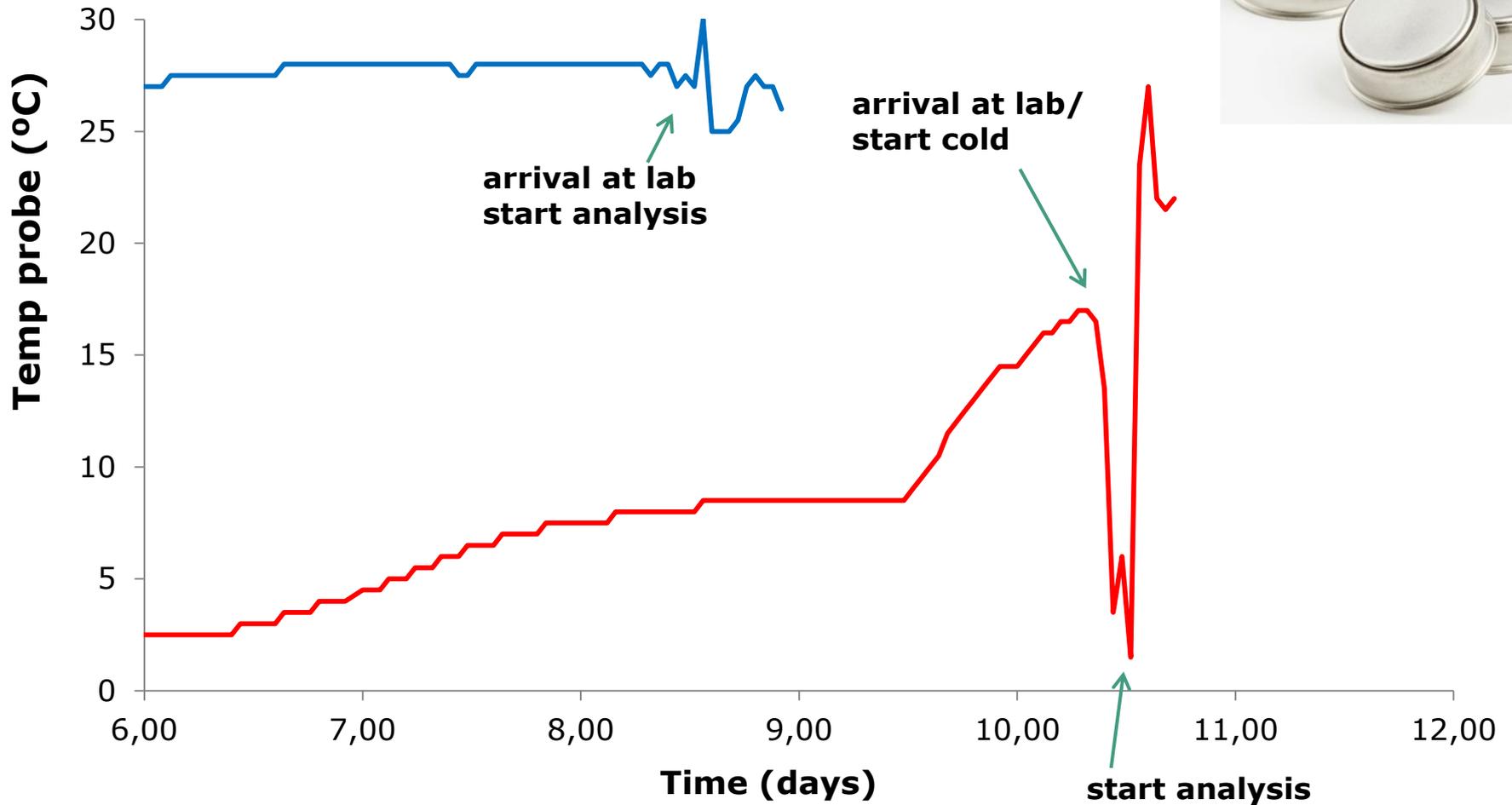
- – 1 NRL from a third country

Transport

➢ Transport time:

- 26 parcels: 0-1 day

- 6 parcels: 2 days

- 2 parcels: 3 days

- 2 parcels: problems at customs

- labcode 35: arrival after 8 days

- labcode 22: arrival after 10 days

➢ Temperature during transport and storage:

- temperature probe between samples

- transport temperature: predominantly -3 °C and + 5 °C

- storage temperature: mostly 0 °C - 6°C.

Temperature samples parcel Lab 35

Temperature chart. Y-axis: Temp probe (°C) from 0 to 30. X-axis: Time (days) from 6,00 to 12,00.

Blue line annotation: **arrival at lab start analysis**

Red line annotations: **arrival at lab/ start cold** and **start analysis**
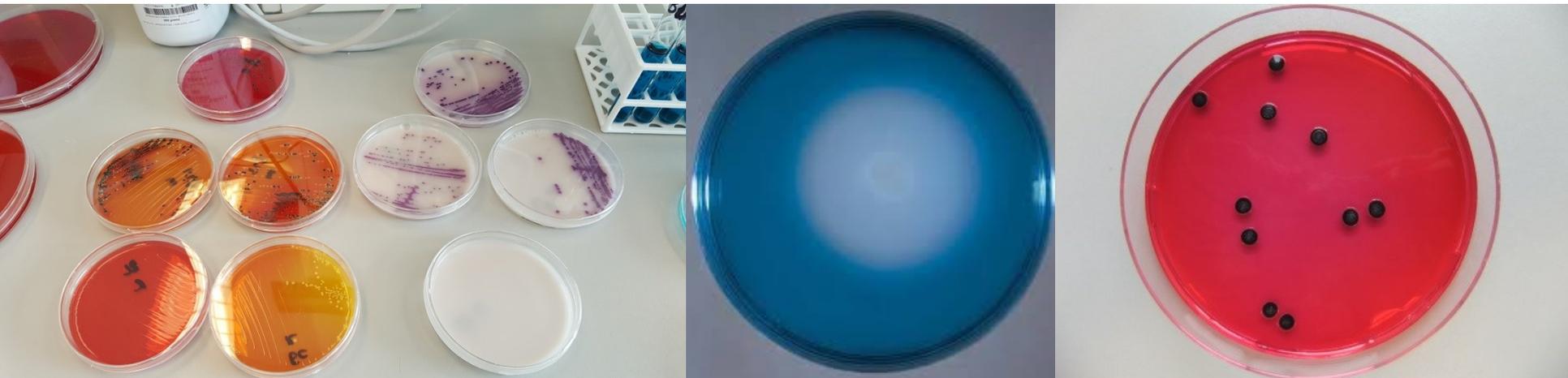
Method PT-PPS

Prescribed: ISO 6579-1:2017

    - Selective enrichment on MSRV

    - Isolation medium XLD & 2nd agar of choice

PT samples: Background flora

Date of testing	Aerobic bacteria cfu/g	*Enterobacteriaceae* cfu/g
Preparation: t = 0 days 17 Sept 2018	4.9×10^8	2.8×10^6
Start analysis t = 14 days 1 Oct 2018 (after storage at 5 °C)	1.2×10^8	1.9×10^5

PT samples: Artificial contamination

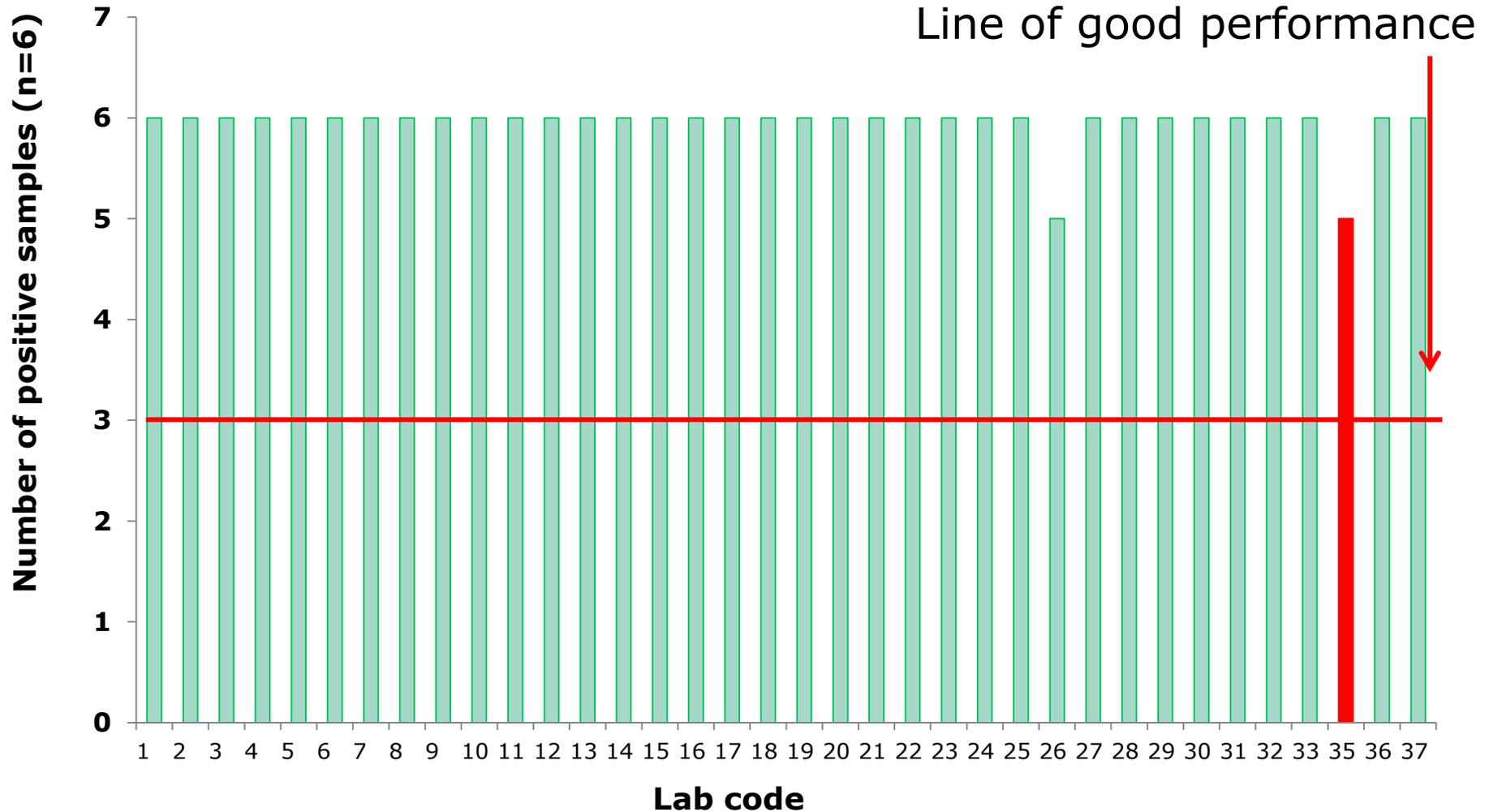| Date of testing	Sample	Low level SI cfu/sample	High level SI cfu/sample
18 Sept 2018	Inoculum of diluted culture	10	53
1 Oct 2018	Inoculated boot socks with chicken faeces, stored 5°C MPN (95% confidence limit)	3.3 (1.1 -10.3)	17.3 (6.5 - 45)

Results: High concentration SI

Results Low concentration SI

Line of good performance

Number of positive samples (n=6) — y-axis scaled 0 to 7

Lab code — x-axis labeled 1 through 37

Samples: specificity, sensitivity, accuracy

Boot sock samples with chicken faeces		All labs n = 35	EU-MS n = 29
Negative samples n=6	No. of samples No. of negative samples Specificity in %	210 210 100%	174 174 100%
Low level (SI) n=6	No. of samples No. of positive samples Sensitivity in %	210 208 99%	174 173 99.4%
High level (SI) n=6	No. of samples No. of positive samples Sensitivity in %	210 209 99.5%	174 173 99.4%
All boot sock samples with SI	No. of samples No. of positive samples Sensitivity in %	420 417 99,3%	348 346 99.4%
All boot sock samples (positive and negative)	No. of samples No. of correct samples Accuracy in %	630 627 99,5%	522 520 99,6%

Control samples: specificity, sensitivity, accuracy

Control samples		All labs n= 36	EU n = 29
Procedure control (moistened boot socks) n = 1	No. of samples No. of negative samples Specificity in %	36 36 100%	29 29 100%
Positive control (Own *Salmonella*) n= 1	No. of samples No. of positive samples Sensitivity in %	36 36 100%	29 29 100%
All control samples n = 2	No. of samples No. of correct samples Accuracy in %	72 72 100%	58 58 100%

Conclusions PT-PPS 2018

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

- *Salmonella* boot sock samples with chicken faeces:
  - ➢ High level SI: 1 lab scored 1 sample negative
  - ➢ Low level SI: 2 labs scores 1 sample negative
  - ➢ Negative samples: all labs scored all samples negative

- Results of lab 35 were not evaluated because of temperature abuse during transport

Thank you all for your participation in this study

EURL *Salmonella*