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

EURL- *Salmonella*

Combined Food-PPS interlaboratory comparison study on the detection of *Salmonella* in hygiene swab samples (2017)

EURL *Salmonella*

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Avian influenza

- Yearly outbreaks Avian Influenza in fall and winter

- Control measures: transport faeces forbidden

- Availability of faeces for pre-test in Nov-Dec and study samples in Februari problematic

Avian influenza

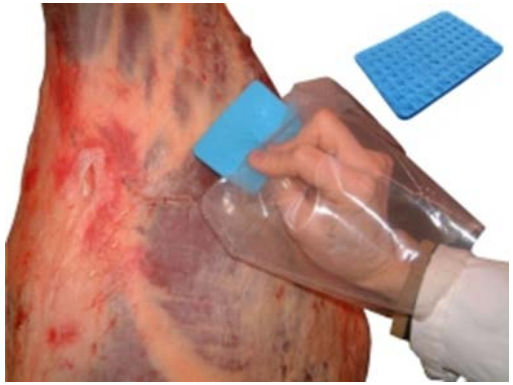
- Change the order of Food and PPS study

Study	Old	New planning
Food/Feed	Sept/Oct	Feb/March
PPS	Feb/March	Sept/Oct
Typing	Nov	Nov

- Transition year 2017: hygiene swabs suitable as food and PPS matrix

Combined EURL-*Salmonella* Detection study Food PPS 2017

- Matrix: Hygiene swabs
- Swab surfaces in food production areas and poultry farms
- Swabs contaminated at EURL
  - background flora
  - high and low concentration of *Salmonella* Typhimurium



Determination background flora

- 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.

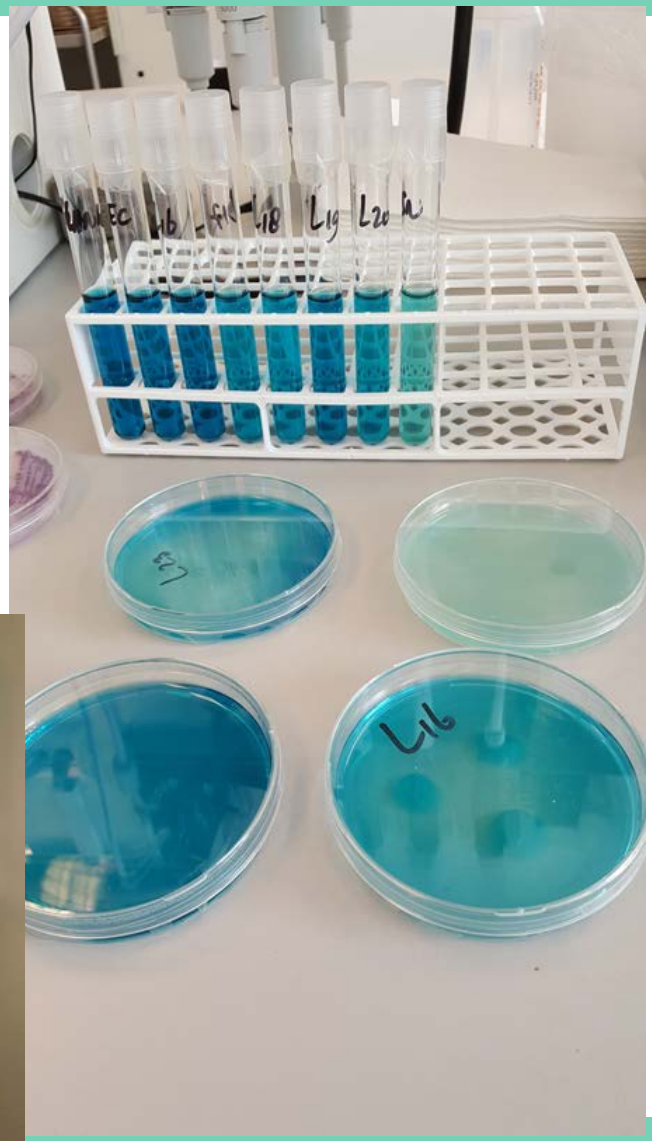
Pre-test: Stability of samples (1)

- 10 ml Peptone Saline Solution to moisten swab
- Add 1 ml of mixture *E.coli* and *C. freundii* (10^6 cfu/ml)
- Add high and low concentration *Salmonella* Typhimurium
  - Low: 5-10 cfu (14 cfu)
  - High: around 50-100 cfu (76 cfu)

Pre-test: Stability of samples (2)

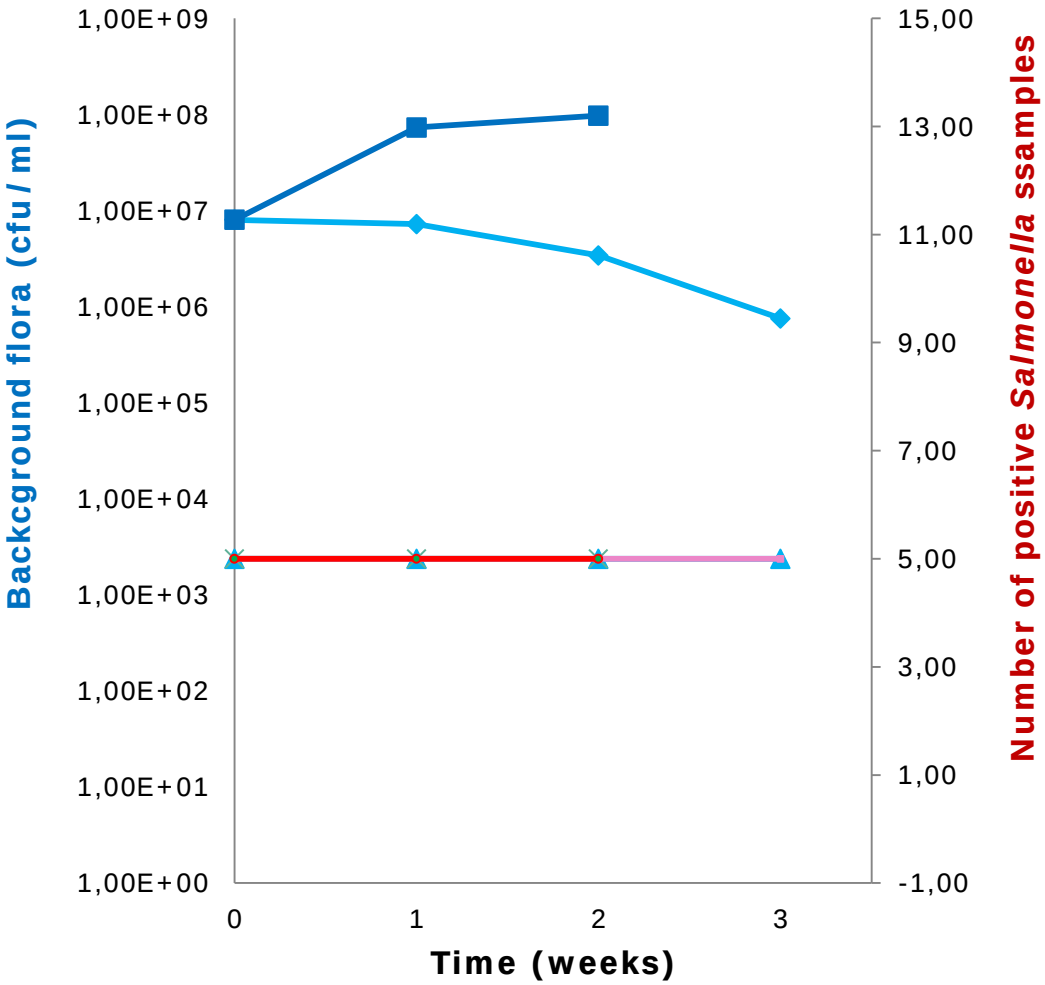
- Storage conditions: 5 °C and 10 °C
  for up to 3 weeks

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



Conclusion Pre-tests

- *Enterobacteriaceae*
  - ~ stable
- *Salmonella*
  - # positive samples stable



Dark: 5°C Light: 10°C

Prolonged storage

	Conc.	Temp (°C)	Time (weeks)			
			0	7	10	13
Background flora	(cfu/ml)	5°C	$6,8x10^6$	$4,7x10^4$	$2,0x10^3$	$5,5x10^5$
STM MSRV	Low (11 cfu)	5°C	100%	6/7	5/7	6/7
STM RVS/MKTTn	Low (11 cfu)	5°C	100%	6/7	5/7	6/7

Study design

- Hygiene swabs + 10 ml PSS
- Background flora: *E.coli/C. Freundii*~10^6 cfu/ml
- Contaminated with *Salmonella* Typhimurium:
  - 6 Low level STM: 5-10 cfu
  - 6 High level STM: 50-100 cfu
  - 6 Blank
- Process controls:
  - C1: Hygiene swab + 10 ml PSS
  - C2: Hygiene swab + 10 ml PSS
    + own positive control

Participants

In total 56 participating NRLs

- 33 NRLs Food and 23 NRLs PPS
- from 28 EU Member States
- 4 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

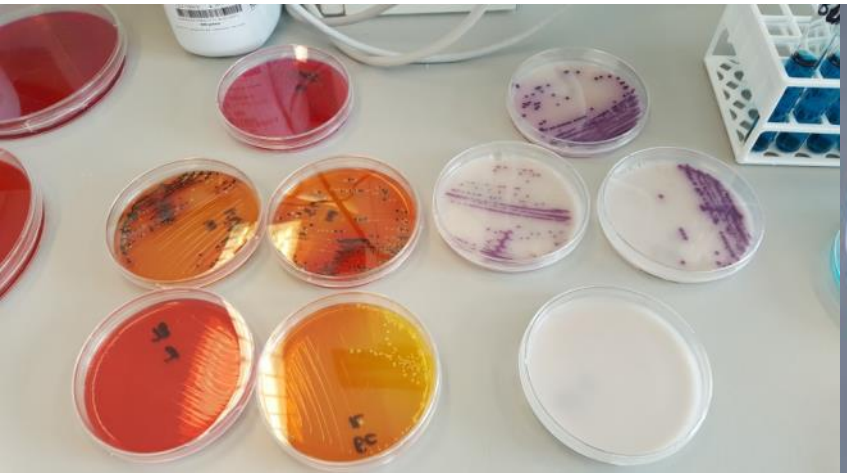
- Transport time:
  - 44 parcels: 0-1 day
  - 11 parcels: 2 days
  - 1 parcels: 3 days

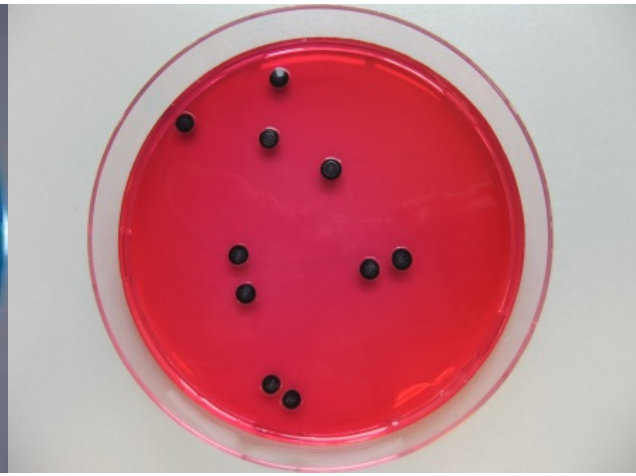
- Temperature during transport and storage:
  - temperature probe in sample bag
  - transport temperature: predominantly -5 °C - 6 °C
  - storage temperature: mostly 0 °C - 10°C.

Method

Prescribed: ISO 6579-1:2017

- Selective enrichment on RVS or MKTTN and/or MSRV
- Isolation medium XLD & 2nd agar of choice



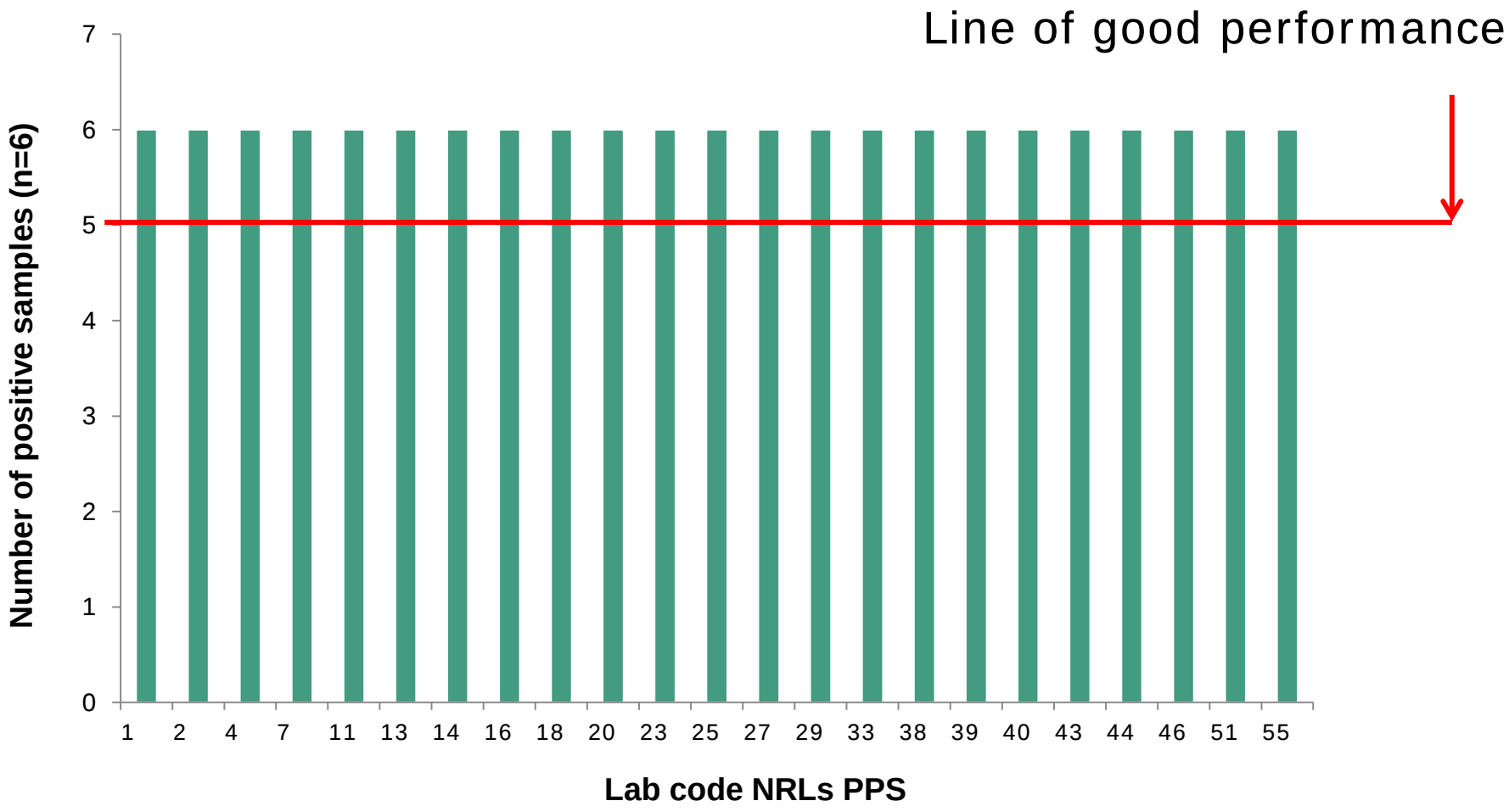


Hygiene swab samples

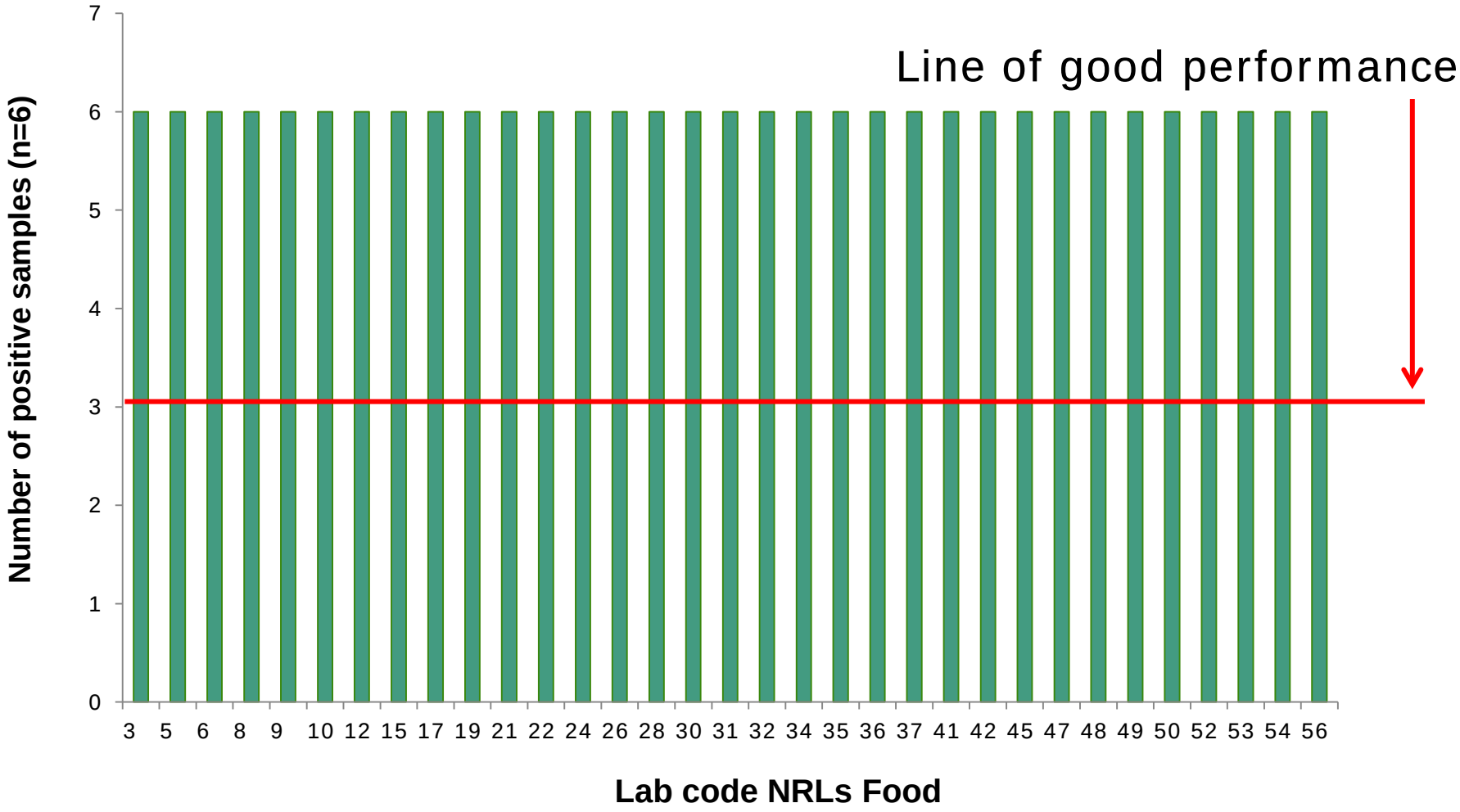
Samples	26 sept 2017		9 Oct 2017 (after storage at 5 °C)		
	Inoculum concentration		Conc. STM MPN (MSRV)	Conc. STM MPN (MKTTn)	*Entero* (cfu / g)
	STM	*Entero* (cfu / g)			
Blanc		7.7×10^5			7.1×10^6
Low	5 cfu	1.3×10^6	7 (2.3-22)	7 (2.3-22)	1.4×10^4
High	107 cfu	7.3×10^7	92 (28-300)	92 (28-300)	4.7×10^6

Results NRLs Food: High concentration STM

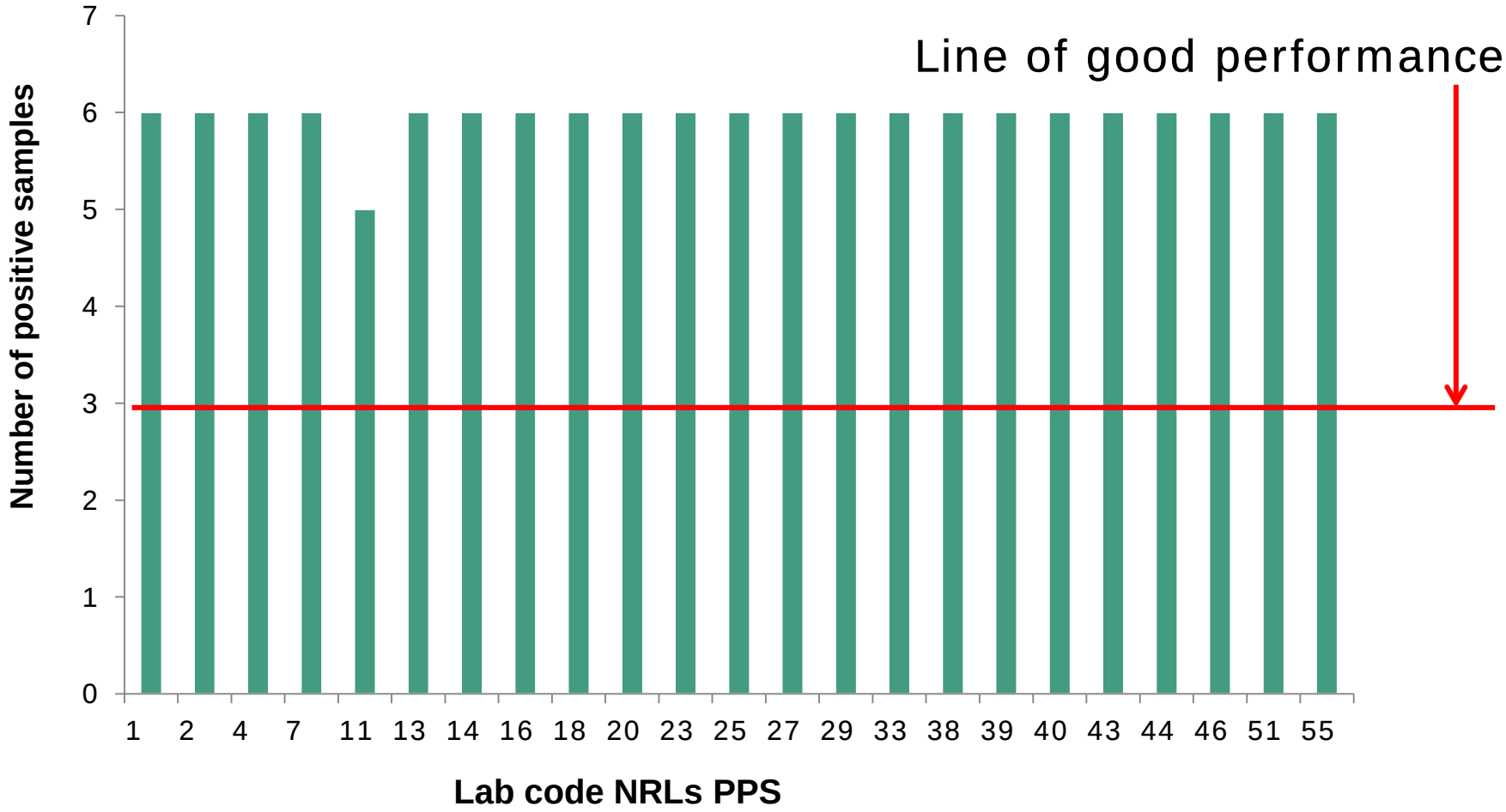
Results NRLs PPS: High concentration STM



Results NRLs Food: Low concentration STM



Results NRLs PPS: Low concentration STM



Samples: specificity, sensitivity, accuracy

Hygiene swabs		Total labs n = 56	NRL-Food n = 33	NRL-PPS n = 23
Blank n=6	No. of samples No. of negative samples	336 334	198 196	138 138 00%
Low level (STM) n=6	No. of samples No. of positive samples Sensitivity in %	336 335 99,7%	198 198 100%	138 137 99,3%
High level (STM) n=6	No. of samples No. of positive samples Sensitivity in %	336 336 100%	198 198 100%	138 138 100%
All swab samples with STM	No. of samples No. of positive samples Sensitivity in %	672 671 99,9%	396 396 100%	276 275 99,6%
All swab samples (positive and negative)	No. of samples No. of correct samples Accuracy in %	1008 1005 99,7%	594 592 99,7%	414 413 99.8%

1 laboratory (24) : 2 positive blanks: poor performance

Control samples: specificity, sensitivity, accuracy

Control samples	Laboratories n = 56	
Procedure control Blank (BPW) n = 1	No. of samples No. of negative samples Specificity in %	56 56 100%
Positive control (Own *Salmonella)* n= 1	No. of samples No. of positive samples Sensitivity in %	56 56 100%
All control samples n = 2	No. of samples No. of correct samples Accuracy in %	112 112 100%

One laboratory (28): error in copying raw data to electronic report: moderate performance

Follow-up study

- Study design:
  - 10 samples
    - 6 blank samples
    - 4 high contaminated samples
  - Concentration:
    - Background flora: 9.6 x 10^6 cfu/ml
    - *Salmonella* Typhimurium: 49 cfu/swab

- 100% correct results

Conclusions combined Food-PPS 2017

- Control swab samples:
  - Excellent performance: 100% correct scores
  - 1 lab (28) moderate performance: error in copying raw data to electronic report

- *Salmonella* swab samples:
  - High level STM: all labs scored all samples positive
  - Low level STM: 55 labs scores all samples positive, 1 labs scored 1 sample negative
  - Blank samples: 55 labs scored all samples negative
  - 1 lab (24) scored 2/6 samples positive: poor performance

- Follow-up study:
  - 100% correct results

Thank you all for your participation in this study

EURL Salmonella