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

EURL *Salmonella* Interlaboratory comparison study FOOD VIII 2016

Detection of *Salmonella* in minced chicken meat

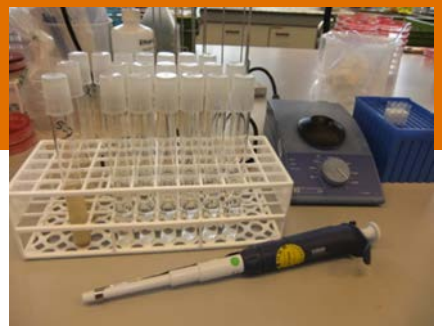
EURL *Salmonella*

Angelina Kuijpers

Time table

Week (2016)	Dates	Subject
38	19 – 23 September	Mailing of the protocol and instructions for the web based test report and link to the NRLs by email.
39	26 September	Mailing of parcels to the NRLs
40	3 October	Performance of the study
43	Before 25 October	Deadline for completing the electronic submission of results: **25 October 2016** (23:59h CET)

EURL *Salmonella* detection study



Comparable to earlier studies

The number of samples to be tested and the type of samples are comparable to the studies organised since 2013:

- Artificial contamination of samples at laboratory EURL-*Salmonella* before sending to NRLs
- Use of webbased testreport
- Less control samples: one blank control & one own positive control
- No Standard Operating Procedure (SOP), Follow EN ISO 6579 (and the underlying EN ISO documents) according to normal routine procedure for detection of *Salmonella* in 'official' samples.
- Reporting results positive or negative per sample

Pre-test: stability of samples (I)

minced meat with *Salmonella*

PRE-TEST

- Artificially contamination of minced turkey meat with a diluted *Salmonella* culture

- *Salmonella* Stanley (SSt) and S. Typhimurium (STM) tested: isolated from human & chicken

- Level of contamination/25 g meat: 10- 15 CFU and 100 CFU

- Stability of samples during: - storage (-20 ºC & 5 ºC)
  - transport (10 ºC)
  - *Salmonella* detection and influence of backgroundflora

- BRO food 2013 storage at NRL 5 ºC high amount of backgroundflora ->if storage at -20 ºC is possible

Pre-test: stability of samples (II)

minced turkey meat

4 samples with SSt6 and STM12 tested

3 weeks at -20 ^{0}C, 5 ^{0}C & 10 ^{0}C

Test 4 samples after

1, 2 and 3 weeks of storage

All samples positive : stable

Backgroundflora:

Enterobacteriacea 10^2 CFU/g

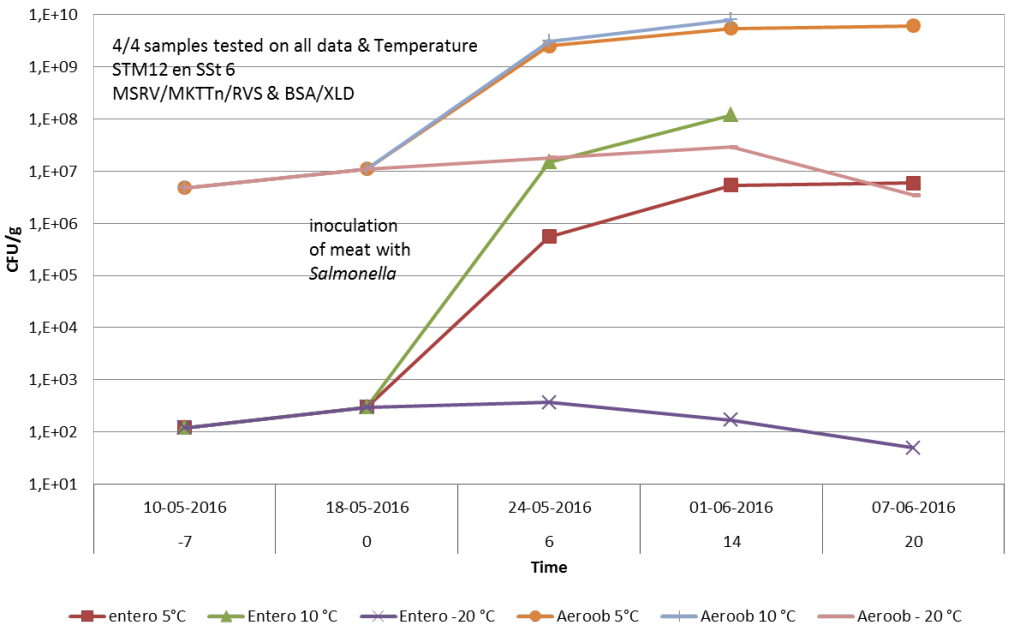
Aerobic 10^7 CFU/g

Increase at 5 ^{0}C & 10 ^{0}C

Entero> log 4-5

Aerobic > log 2-3

Stable for 3 weeks at -20 ^{0}C



Pre-test: stability of samples (III)

minced turkey meat with SSt

6 samples with SSt12 and SSt42 tested

3 weeks -20 ^{0}C

2 weeks at -20 ^{0}C, 1 week 5 ^{0}C

1 week -20 ^{0}C, 1w 5 ^{0}C, 1w -20 ^{0}C

All samples positive : stable

Backgroundflora:

Enterobacteriacea 10^3 CFU/g

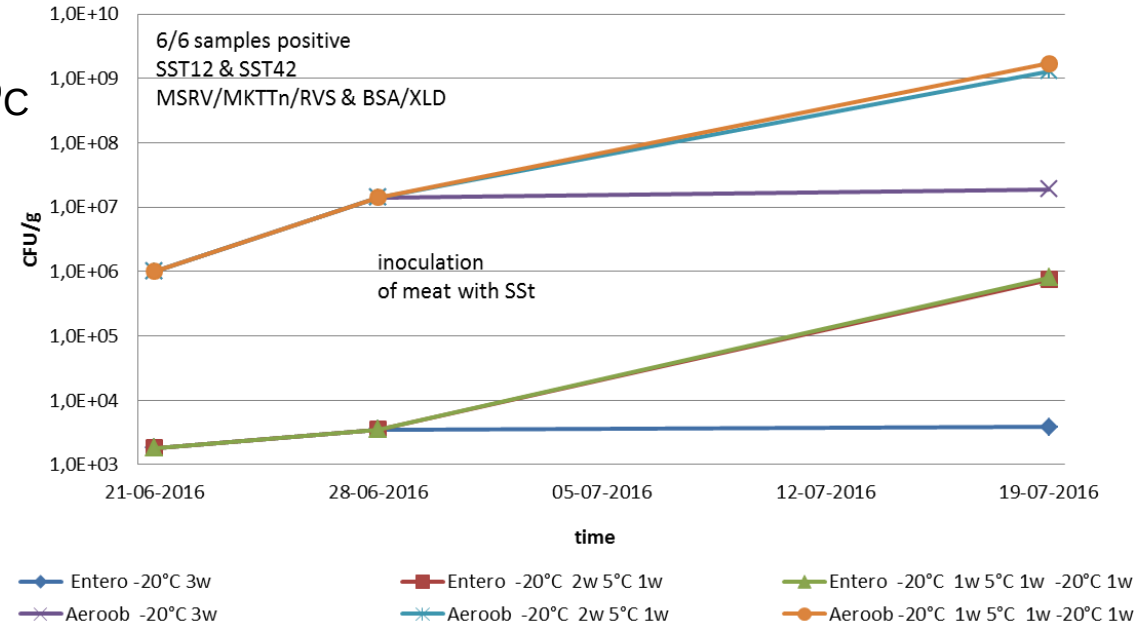
Aerobic 10^6 CFU/g

Stable at -20 ^{0}C

Increase at 5 ^{0}C

Entero > log 3

Aerobic > log 2



Pre-test: stability of samples (IV)

minced meat with *Salmonella* Stanley

CONCLUSION

- Matrix: minced turkey meat

- Artificial contamination of meat with a diluted culture :
  - SSt low level (15 -20 CFU/25g meat)
  - SSt high level (50 -100 CFU/25g meat)
  - Blank

- After contamination of the meat with SSt

  Storage at the EURL and the NRLs at -20 ^{0}C

But at the very last minute..

change of matrix because of naturally contamination of minced turkey meat
New Matrix: minced chicken meat

Study design

Artificially contaminated	Samples (n=18): 25 g minced meat
S. Stanley low	6
S. Stanley high	6
Blank	6

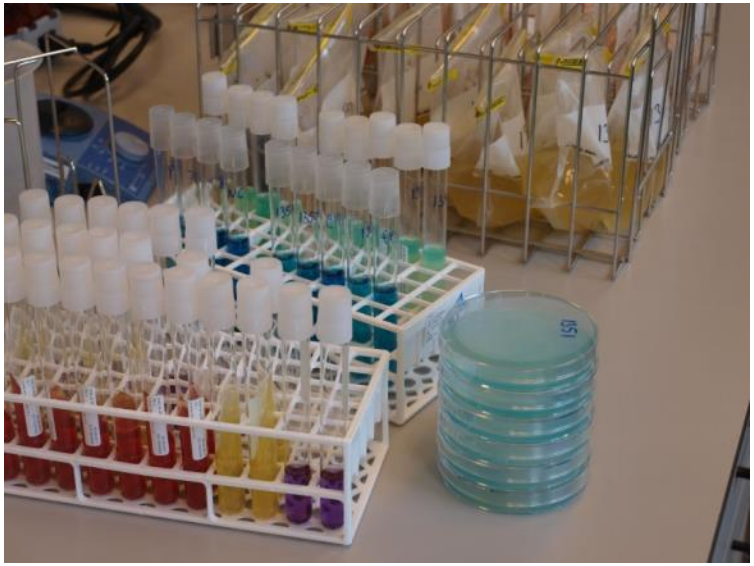
Number and level of samples according to ISO/TS 22117 (2010)

Control samples	n=2
Procedure control blank: BPW	1
Positive control: Own control with *Salmonella*	1

Analytical methods in study

- Follow FDIS ISO 6579-1 (choose between RVS and MSRV)

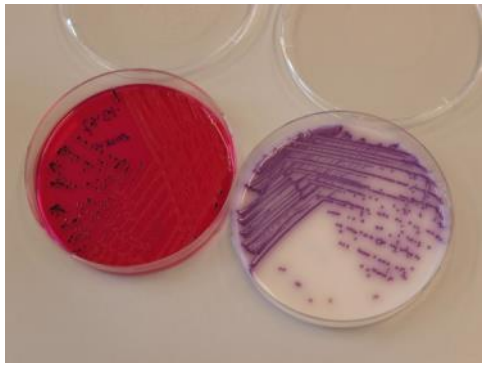
- Selective enrichment in : MKTTn and RVS or
  MKTTn and MSRV or
  MKTTn, RVS and MSRV



- Isolation medium

- XLD and 2nd agar for choice

- Confirmation



Participants

34 Laboratories

- 30 NRLs from 28 EU-Member States (MS)

- 4 NRLs from third countries

-EU candidate MSs or
potential EU candidate MSs
-members of EFTA countries
(European Free Trade Association)
-non-European countries

Transport of samples

Cooling devices & Temperature logger

Packages all transported as Biological Substances Cat. B UN3373 by DHL door-to-door

- **Transport time**:
  - 27 parcels — **1 day**
  - 6 parcels — **2 days**
  - 1 parcel (non-EU MS) — **3 days** delay at the customs

Transport of samples

Cooling devices & Temperature logger

Packages all transported as Biological Substances Cat. B UN3373 by DHL door-to-door

Temperature during

-**transport**: all parcels /NRLs at least **-1 ºC**

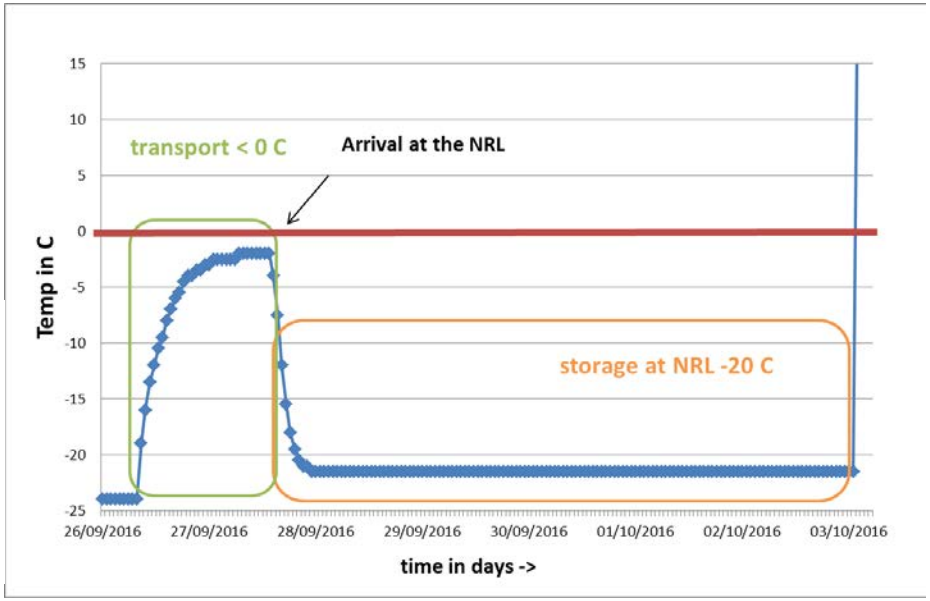
-storage at the NRL

31 NRLs between **-15 ºC and - 28 ºC**

1 NRL **-7 ºC**

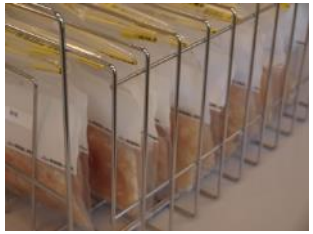
1 NRL **1 ºC**

1 NRL **room Temp** after arrival ?



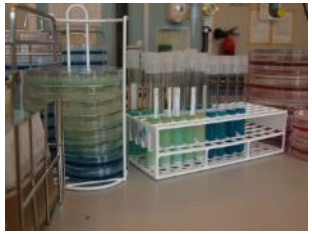
Material & Methods

Follow as much as possible your routine procedure



- Addition of 225 ml BPW to plastic bags (samples)

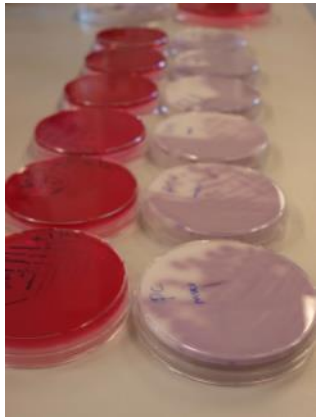
  (BPW at least at room temperature)



- Homogenisation

  (according ISO 6887-2)

- Incubation BPW sample at 37 ºC for 18 h

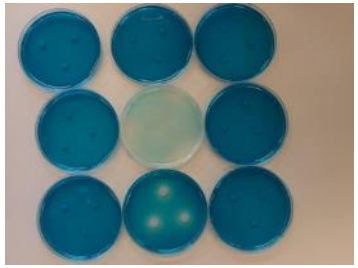


- Selective enrichment media MKTTn and RVS and/or MSRV

- Isolation on XLD and 2nd plate & Confirmation

Level of contamination *S.* Stanley

Date of testing	Low level SST CFU / sample 25 g	High level SST CFU / sample 25 g
21 September 2016 Inoculum of meat	16	73
3 October 2016 Meat inoculated with SSt and stored at - 20 ^{0}C for 10 days	35 MPN (11-110)	55 MPN (16-188)

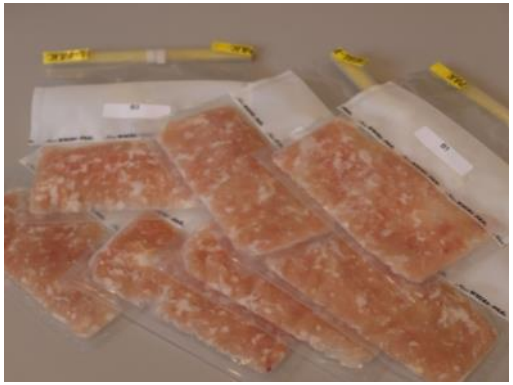


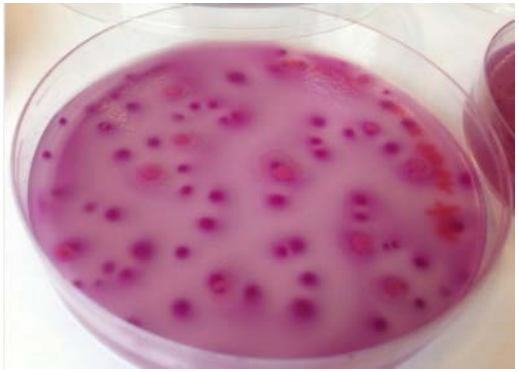
Performance of the study 3 October 2016
MPN: Most Probable Number (5 tube), (95 % confidence interval)

Backgroundflora minced chicken meat

Date of testing	Enterbacteriaceae CFU / g	Aerobic bacteria CFU / g
3 October 2016 After storage for 3 days at +5 °C followed by 10 days at -20 °C	$4 * 10^4$	$2 * 10^6$

Performance of the study 3 October 2016





ISO 17025
ACCREDITED LABORATORY

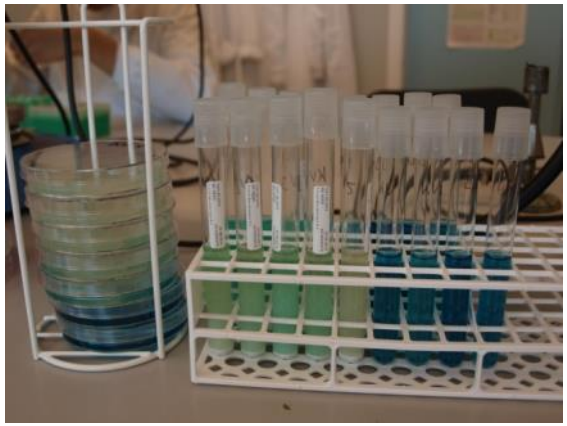
Accreditation according to ISO/IEC 17025

All 34 participants are accredited

Accredited method:

32 NRLs ISO 6579: 28 NRLs also Annex D of ISO 6579

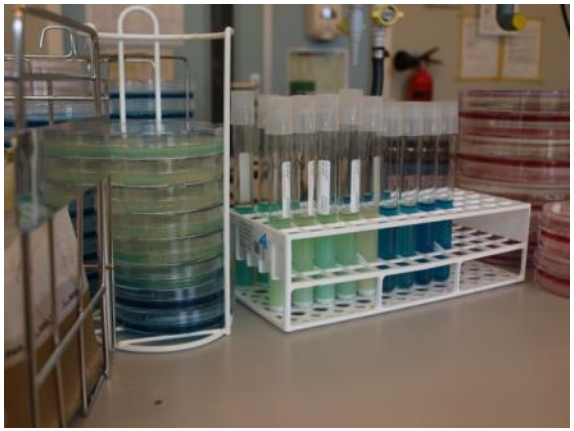
2 NRLs (non- EU-MS) only Annex D of ISO 6579



ISO International Organization for Standardization

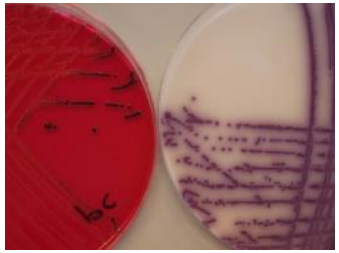
Media/ Methods used

- Media for selective enrichment:
  - 19 NRLs MKTTn, RVS & MSRV (2015:27)
  - 6 NRLs MKTTn & RVS
  - 9 NRLs MKTTn & MSRV (2015:3)



- Media for 2nd isolation medium:

-BGA mod	8
-Rambach	6
-BPLS & RS	5
-BGA	3
-SM(ID)2& BSA	2
-ASAP, Compas S, Chrom S	1



- Additional own method: PCR 9 participants (8 real time PCR)

Criteria for Good performance

minimum result

Samples (n=18) Minced meat	Percentage positive	No of positive samples/ Total No. Of samples
S. Stanley low	50 %	3/6
S. Stanley high	80 %	5/6
Blank	20 % at max	1/6 at max

Control samples (n=2)		
Procedure control blank: BPW	0 %	0/1
Positive control: Own control with *Salmonella*	100%	1/1

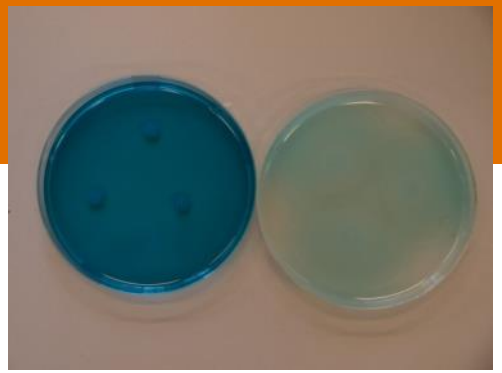
Control samples

The laboratories used their own routine positive control.

Positive controls:	diluted culture	20
(1 -10^4 CFU/sample)	lenticule disc	7
	freeze dried ampoule	4
	culti-loop, kwik-stik, Vitroid	1

Salmonella serovar.	*S*. Enteritidis	14
	S. Typhimurium	7
	S. Nottingham	5

S. Blegdam, S. Abaetetuba, S. Senftenberg, S. Dublin
S. Bongori, S. Tennessee, S. Harleystreet, S. Alachua



Results control samples

Procedure control Blank (BPW)

- All NRLs analysed this sample correctly negative

Positive control (own) with *Salmonella*

- All NRLs analysed this sample correctly positive

Correct scores (%)

Control samples

	MKTTn and RVS or / and MSRV XLD or 2nd plate
Procedure control (BPW)	100
Positive control (own)	100
All control samples	**100**

Results meat samples (I)

Blank

- 31 NRLs scored all 6 blank meat samples correctly negative.
- 3 laboratories found 1 sample positive for *Salmonella.*

Low SSt

- All NRLs detected *Salmonella* in all 6 samples.

High SSt

- 33 NRLs detected *Salmonella* in all 6 samples.
- 1 laboratory found 1 sample negative for *Salmonella.*

All participants scored a good performance !!!!

Results meat samples (II)

Blank

- 3 laboratories found 1 sample positive for *Salmonella.*

High SST

- 1 laboratory found 1 sample negative for *Salmonella.*

(and 1 blank sample positiv for *Salmonella*)

- *exchange, cross-contamination, misinterpretation of the results ??*
- *S*. Infantis isolated in 3 Blank samples.

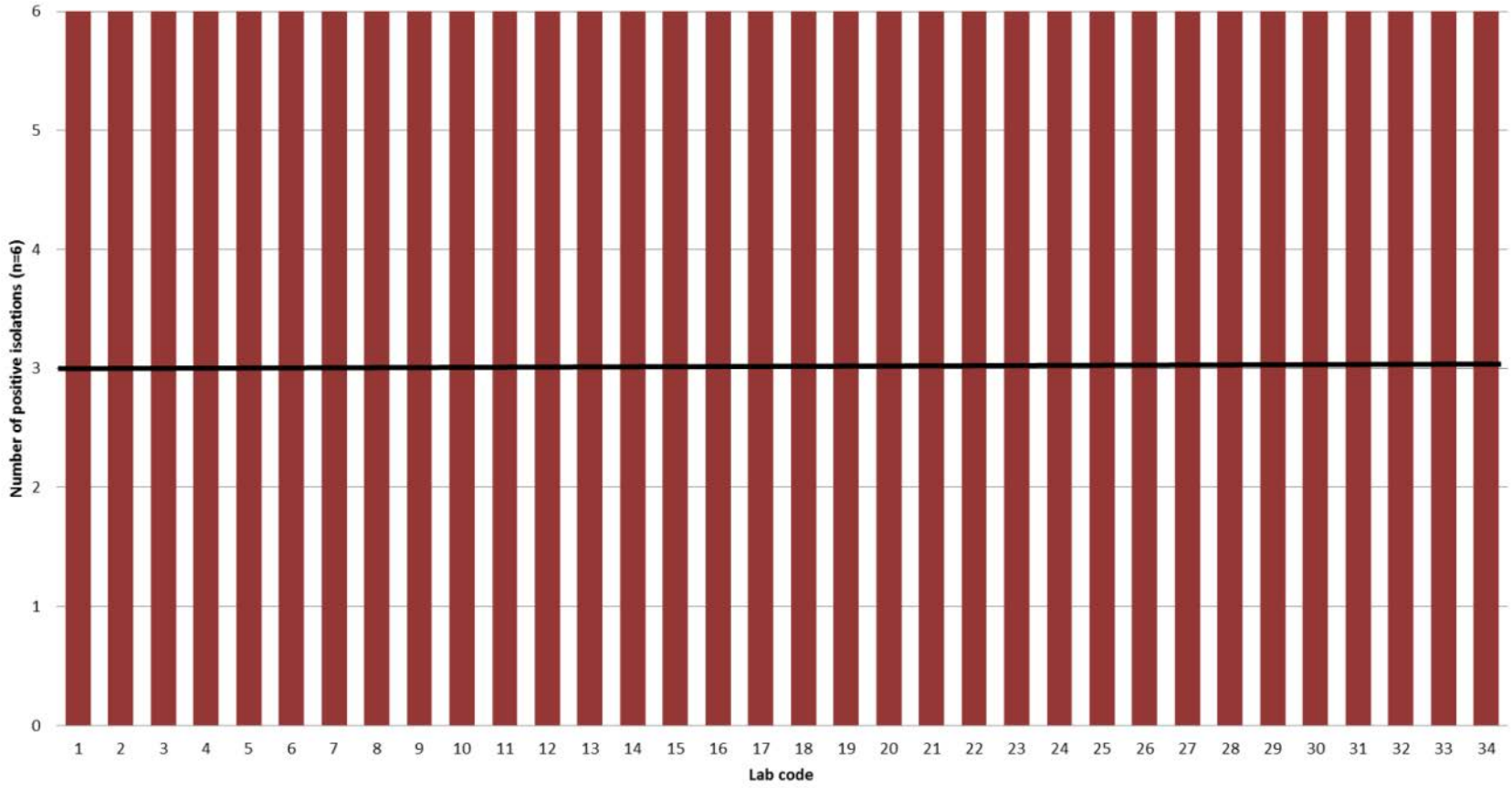
No other S. Infantis at the laboratories (positive control, samples…)

Possible clarification:

Naturally contamination of meat with *S*. Infantis at a very low level because all other tested Blanks by NRLs and EURL (>200) were tested negative.

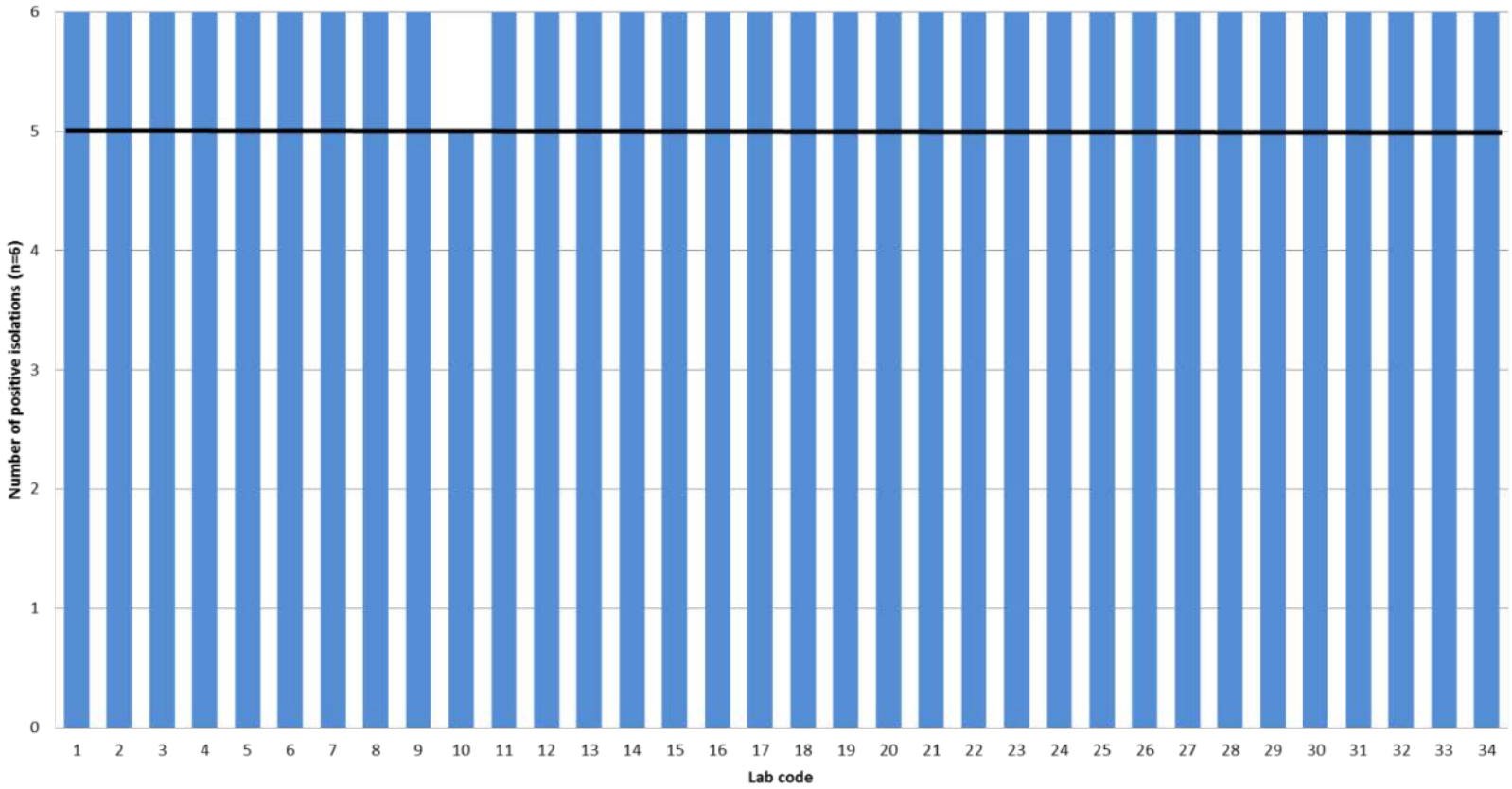
Results minced chicken meat samples (III)

Low level SSt



Results minced chicken meat samples (IV)

High level SSt



Specificity, sensitivity & accuracy (%)

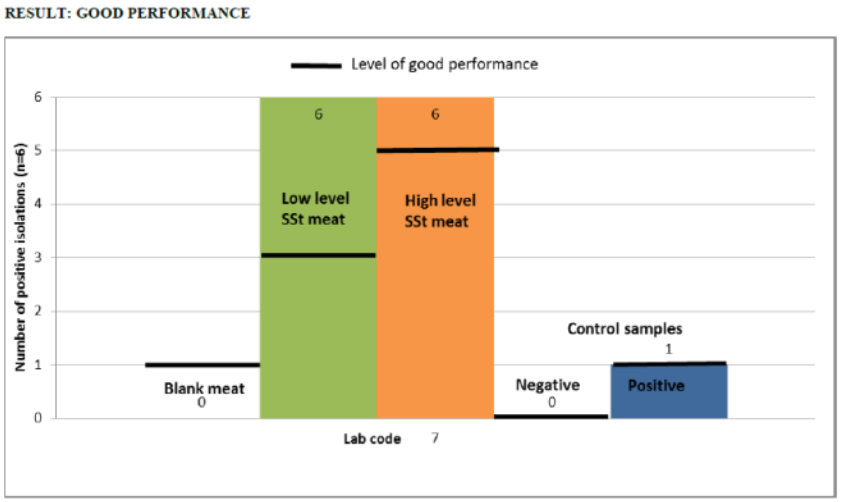
minced chicken meat samples

		MKTTn and RVS or/and MSRV XLD or 2nd plate
Specificity	Blank	99
Sensitivity	Low SSt	100
	High SSt	99
	All SSt samples	99
Accuracy	All samples	**99**

Performance of the NRLs

- All 34 NRLs achieved

the level of good performance



Suggestion from participant

- Stomacher bags with filter to contain the samples.

Conclusions

- All participants achieved the level of **good performance**

- The **accuracy** rates for the artificially contaminated minced chicken meat samples were high **99 %.**

EURL Salmonella

THANKS for your participation & cooperation in this study

Angelina Kuijpers