Rijksinstituut voor Volksgezondheid
en Milieu
Ministerie van Volksgezondheid,
Welzijn en Sport

Preliminary results EURL-*Salmonella* Proficiency Test food 2021

Detection of *Salmonella* spp. in liquid whole egg

Robin Diddens

EURL-*Salmonella* workshop May 2021

EURL-*Salmonella* PT food 2021

- Matrix: liquid whole egg
- Proficiency Test in 2015
  - *S.* Enteritidis from chicken product
  - Artificially contaminated with a high and low level
  - Stable samples

Salmo

SCIENTIFIC REPORT

EFSA Journal

APPROVED: 19 January 2021

doi: 10.2903/j.efsa.2021.6406

Surveillance en monitoring van Salmonella bij de mens, landbouwhuisdieren en in voedsel in Nederland, 2019

Bronnen van besmetting

De geschatte bijdragen aan het voorkomen van salmonellose bij mensen door reizen, landbouwhuisdieren en hun producten, worden getoond in figuur 2. Het bronattributiemodel schat de verdeling van het voorkomen van de verschillende van serovars bij de mens op basis van de verdeling van het voorkomen in de verschillende bronnen. Daarbij betrekt men ook het geconsumeerde volume, de besmettingsgraad en de fractie van het voedsel wat rauw of goed doorbakken wordt geconsumeerd (5). In tegenstelling tot de historisch laagste schatting van aan ei gerelateerde infecties in 2018 (18%) ten opzichte van de afgelopen 35 jaar, zien we in 2019 een stijging van het aantal aan ei gerelateerde infecties tot 26%. Dit kwam vooral door het relatief hogere aantal gemelde S. Enteritidis-infecties in 2019. Het aandeel van varkens als bron is nog steeds het hoogst en was in 2019 28%. Dit is lager dan die in 2018 (34%) en werd voornamelijk veroorzaakt door het lagere aantal gemelde S. Typhimurium-infecties. Slechts 2% was afkomstig van rund en 6% van kip. Ongeveer 7% van de *Salmonella*-infecties werd veroorzaakt door contact met reptielen (*Salmonella* is een commensale darmbacterie bij reptielen). Van ~19% kon de bron niet worden geschat of was de *Salmonella*-infectie in het buitenland opgelopen.

For th
'eggs and
8.7% by
are in the

0% were caused by
oducts thereof' and
outbreaks for 2019

Cereals, dried seeds	13	878	8 (0.91)	16	3,149	79 (2.51)
Other processed food products and prepared dishes	12	1,356	1 (0.07)	19	12,989	16 (0.12)

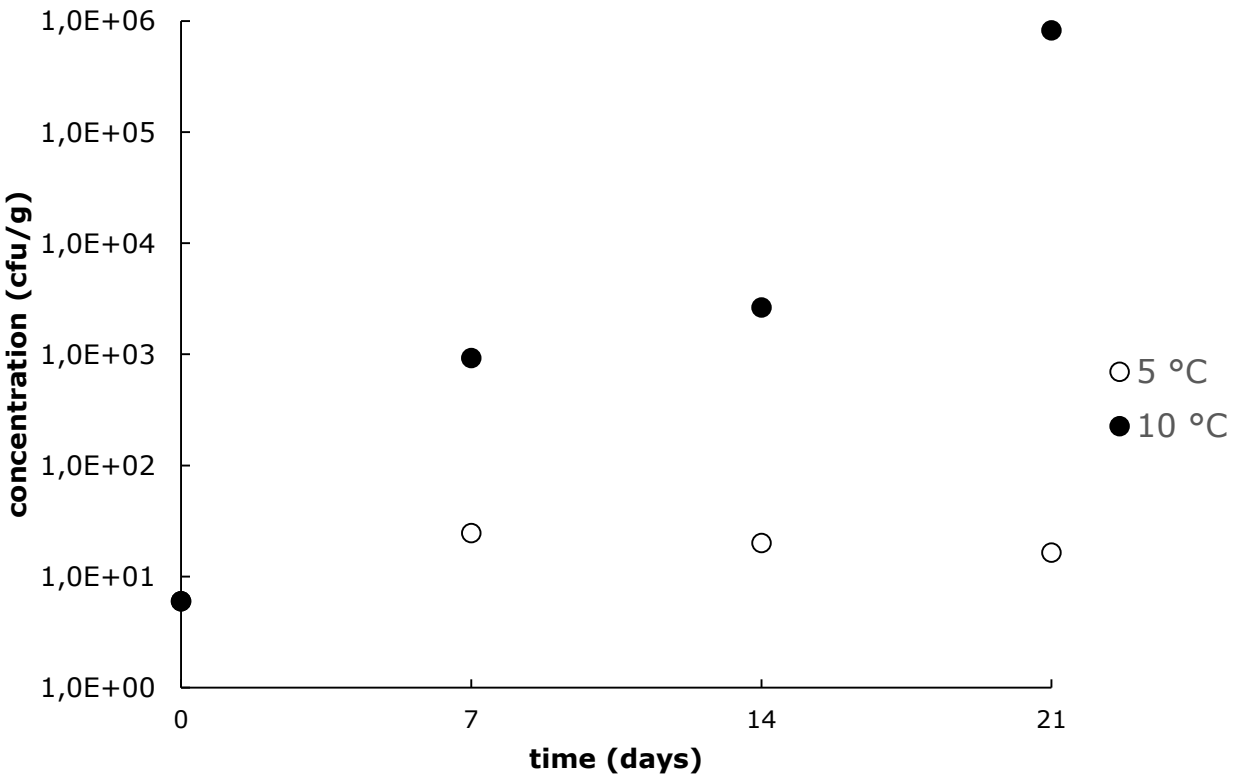
N: number
(a): Meat consisting of ground meat other than beef and pork mixed together.

PT food 2021 – pre-test

- 1 kg package of liquid whole egg (pasteurised)
  - *Salmonella* free
- Test of natural background flora
  - aerobic count & *Enterobacteriaceae*
- Spike with different concentrations *S.* Enteritidis (SE)
  - 4 cfu and 8 cfu
- Storage temperatures: 5 °C and 10 °C
- Testing at t = 0, 7, 14 and 21 days
- 6 samples per conc. SE, storage T and testing day

Pre-test background flora

Aerobic count

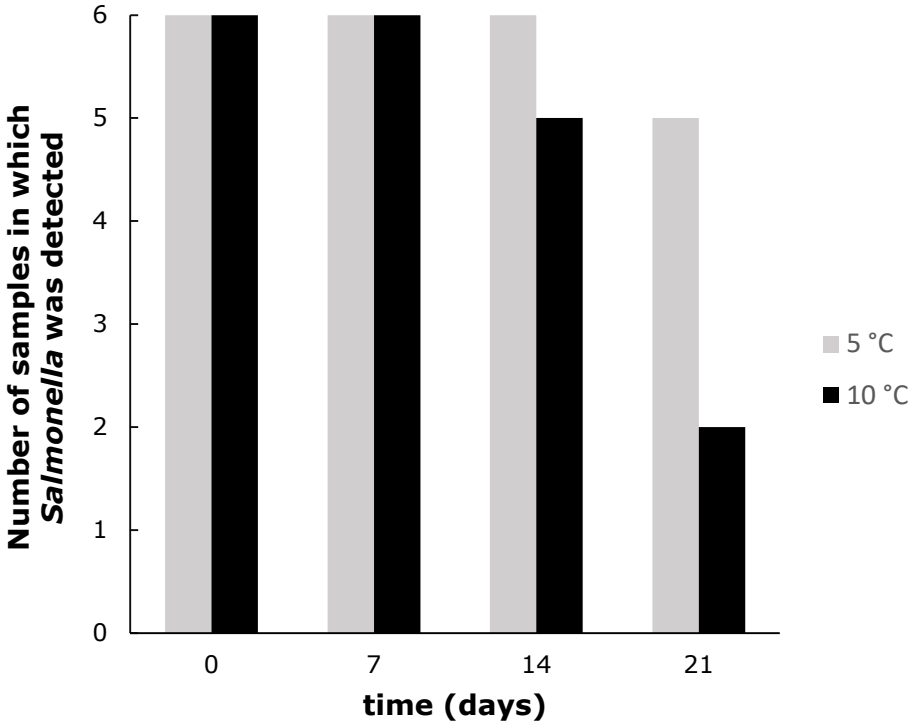


Enterobacteriaceae

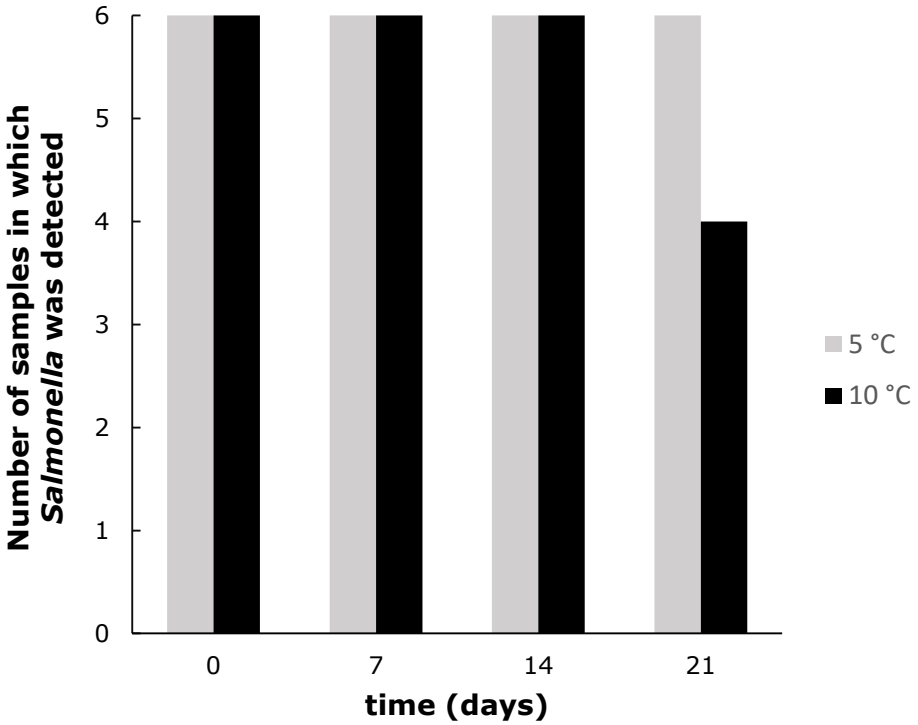
<1 cfu/g

Pre-test *Salmonella*

Spike with 4 cfu SE



Spike with 8 cfu SE



Proficiency Test design

- Samples: 25 g liquid whole egg
- *Salmonella* Enteritidis

- 14 samples (A1 – A14):
  - 4 negative samples (no *Salmonella* added)
  - 6 samples with a low level of *Salmonella* Enteritidis
    - Inoculation 10 cfu / 25 g liquid whole egg
  - 4 samples with a high level of *Salmonella* Enteritidis
    - Inoculation 100 cfu / 25 g liquid whole egg

- 2 control samples
  - Sample CTRL1: procedure control (only BPW)
  - Sample CTRL2: own positive control (with or without matrix)

NEN-EN-ISO 6579-1:2017

INTERNATIONAL STANDARD

ISO 6579-1

First edition 2017-02

Method(s)

- Prescribed method
  - EN ISO 6887-4:2017
    - Protocol for the preparation of samples
  - EN ISO 6579-1:2017(/Amd 1:2020)
    - BPW
    - Selective enrichment: MKTTn & RVS and/or MSRV
    - Selective isolation media: XLD and second choice
    - Confirmation
- Second detection method
  - Not used to asses performance

Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* —

Part 1:
Detection of *Salmonella* spp.

Microbiologie de la chaîne alimentaire — Méthode horizontale pour la recherche, le dénombrement et le sérotypage des Salmonella —

Partie 1: Recherche des Salmonella *spp.*

Participants

- 33 laboratories participated

- 28 NRLs from EU-Member States (MS)
- 5 NRLs from third countries

  EU candidate MS, members of the European Free Trade Association (EFTA), and United Kingdom

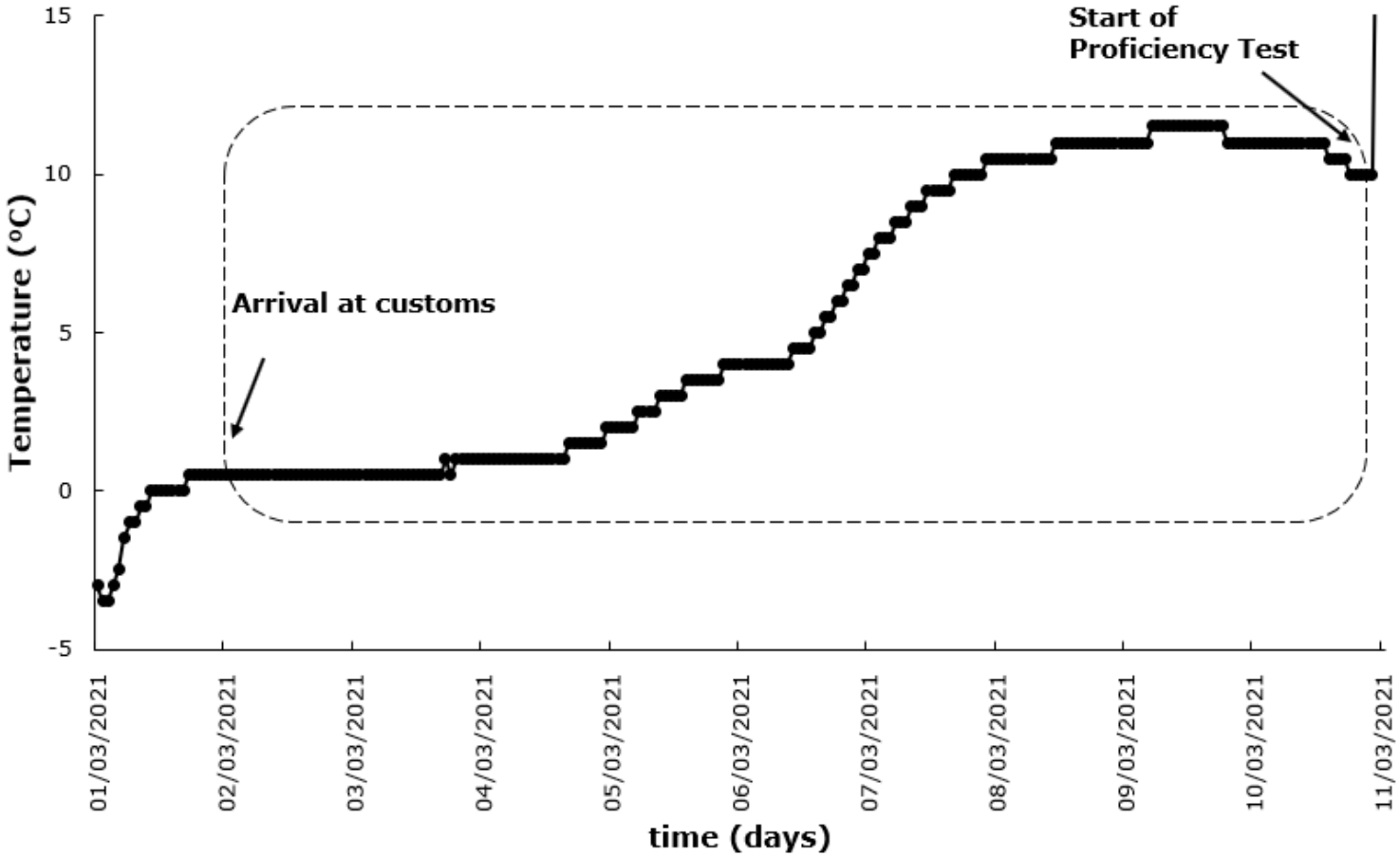
Temperature during transport and storage

- Samples shipped with frozen cooling elements

- 32 parcels were delivered within 1 to 2 days at the NRLs
  - Transport below 5 °C
  - Storage between 0 °C and 7 °C

- 1 parcel was delivered after 9 days of transport
  - Held at customs

Temperature during transport and storage



Natural background of liquid whole egg

Date	Aerobic bacteria (cfu/g)	*Enterobacteriaceae* (cfu/g)
16 February 2021	$9,6 \times 10^2$	<1
9 March 2021[a]	$3,2 \times 10^3$	<1

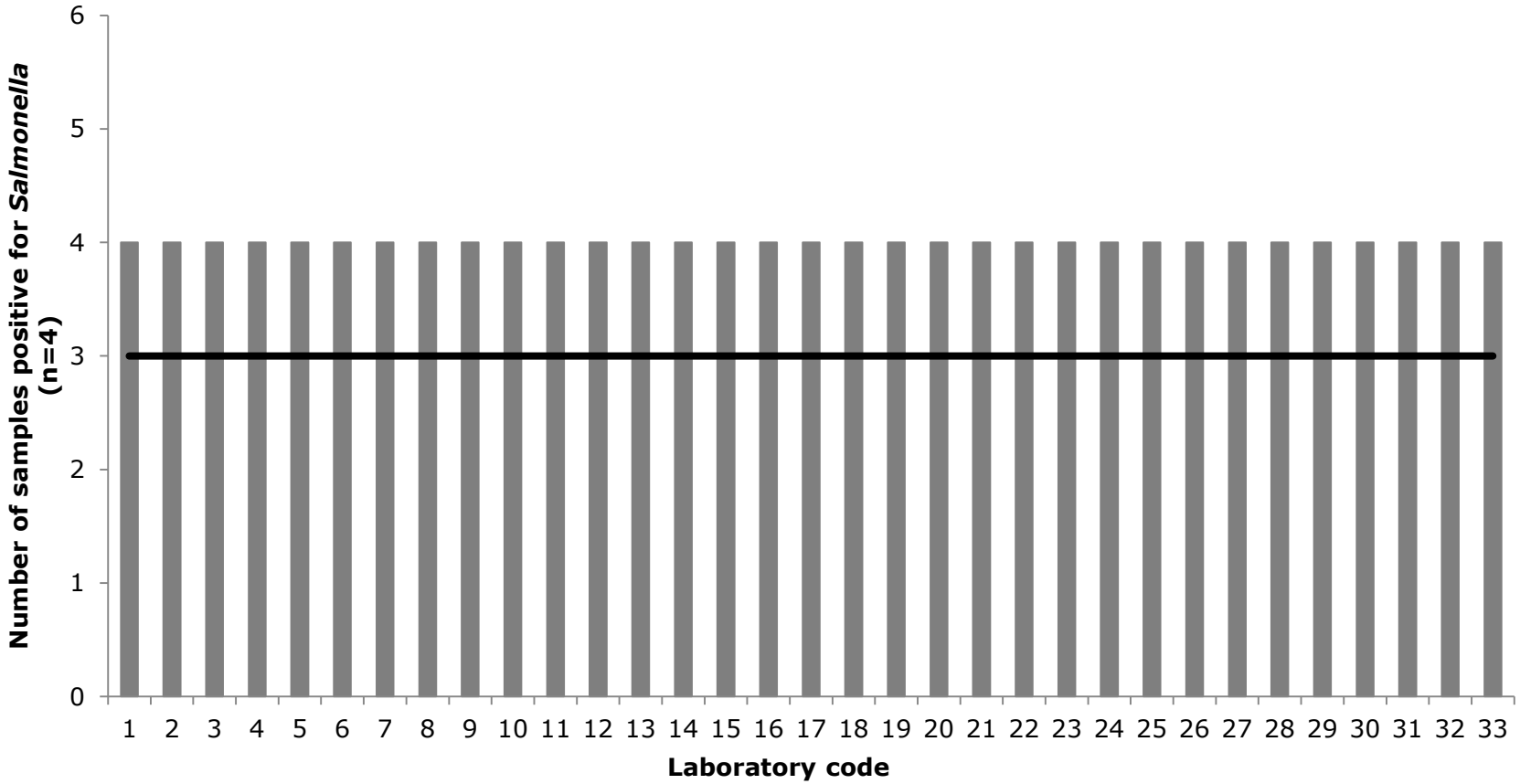
[a] After storage at 5 °C for 3 weeks

Artificial contamination

Date	Low level SE cfu per sample	High level SE cfu per sample
25 February 2021 Inoculation of liquid whole egg	10	69
8 March 2021[a] MPN of liquid whole egg samples, inoculated with SE (95% confidence limit)	3,3 (1,1 – 10,3)	160 (52,5 – 500)

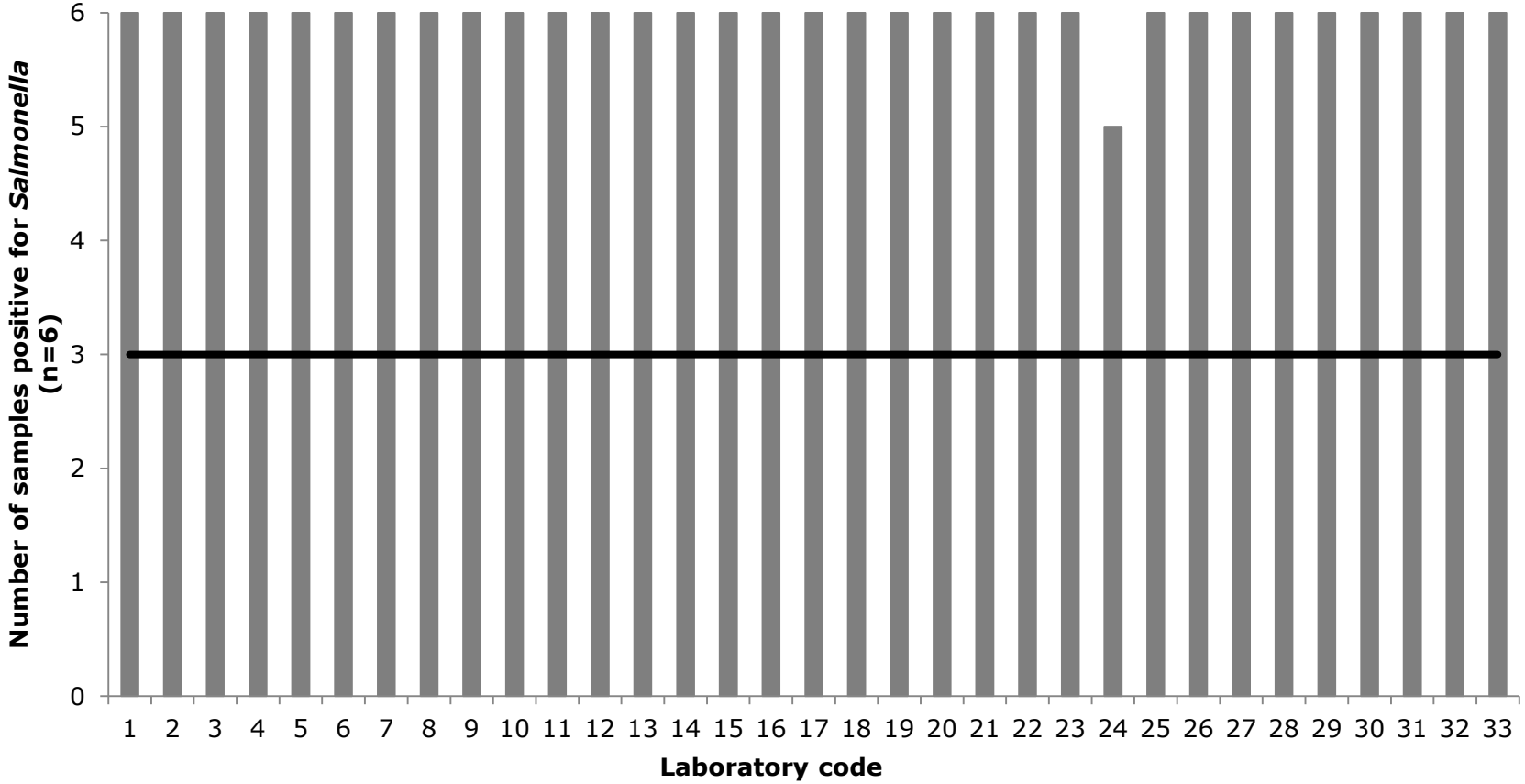
[a] After storage at 5 °C for 1,5 week

Results – high level *S*. Enteritidis



:level of good performance

Results – low level *S*. Enteritidis



:level of good performance

Results

Samples		All participants n = 33
High level of *Salmonella* Enteritidis n = 4	No. of samples	132
	No. of positive samples	132
	Sensitivity in %	100%
Low level of *Salmonella* Enteritidis n = 6	No. of samples	198
	No. of positive samples	197
	Sensitivity in %	99,5%
Negative samples n=4	No. of samples	132
	No. of negative samples	132
	Specificity in %	100%
All liquid whole egg samples artificially contaminated with *Salmonella*	No. of samples	330
	No. of positive samples	329
	Sensitivity in %	99,7%
All liquid whole egg samples	No. of samples	462
	No. of correct samples	461
	Accuracy in %	99,8%

Results

Control samples		All participants n = 33
Procedure control (BPW only) n=1	No. of samples No. of negative samples Correct score in %	33 33 100%
Positive control with *Salmonella* n=1	No. of samples No. of positive samples Correct score in %	33 33 100%
All control samples n=2	No. of samples No. of correct samples Accuracy in %	66 66 100%

All laboratories fulfilled the criteria of **good performance**!

Technical deviations

	BPW	MKTTn			RVS		MSRV		
Laboratory code	**hours incu-bation**	**Conc. novobiocin (mg /L)**	**pH**	**T (°C)**	**pH**	**T (°C)**	**Conc. novobiocin (mg/L)**	**pH**	**T (°C)**
EN ISO 6579-1 (/A1:2020)	18 ± 2 hours	40 mg /L	7 - 8,2	34 °C - 38 °C	5,2 ± 0,2	41,5 °C ± 1 °C	10 mg / L	5,1 - 5,4	41,5 °C ± 1 °C
9	24	40	?	37	?	41,5			
13	19	10	8,02	37	5,42	41,5	10	5,42	41,5
14	18	40	6,6	36	5,2	41,5			
18	18	40	6,80	37,0	5,40	41,5			
29	18	40	8,08	37				5,38	37
30	18				5,2	42	10	5,3	42
31	19	0	7,80	41,2	5,30	41,2			

Positive control

***Salmonella* serovar**	**Number of labs**
S. Enteritidis	9
S. Typhimurium	8
S. Nottingham	5
S. Abaetetuba	3
S. Agona	1
S. Alachua	1
S. Blegdam	1
S. Derby	1
S. Harleystreet	1
S. Poona	1
S. Tranoroa	1
Salmonella bongori serovar 66 : z41: -	1

Advisable to use a serovar rarely isolated from routine samples
Possible cross-contamination can be detected more easily

Positive control

Salmonella serovar	Number of labs
S. Enteritidis	9
S. Typhimurium	8
S. Nottingham	5
S. Abaetetuba	3
S. Agona	1
S. Alachua	1
S. Blegdam	1
S. Derby	1
S. Harleystreet	1
S. Poona	1
S. Tranoroa	1
Salmonella bongori serovar 66 : z41: -	1

Concentration *Salmonella* (cfu/sample)	Number of laboratories
≤10	10
11-29	5
30-130	10
131-500	2
$\geq 10^4$	4
Not defined	2

Media capable of supporting the growth of the target organisms in low numbers?

Second detection method

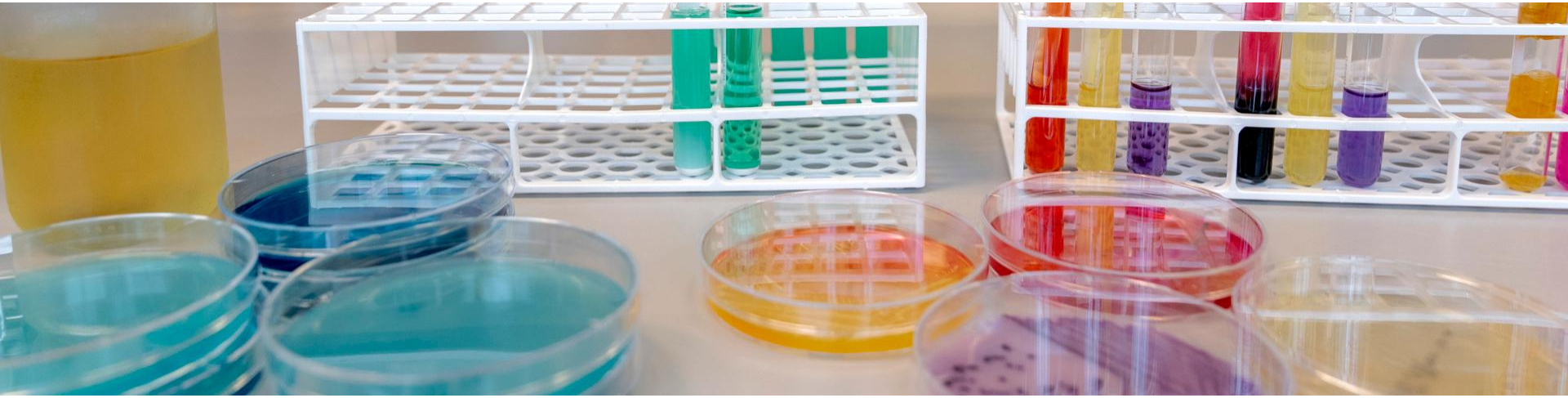
- Used (routinely) in laboratories to analyse samples
  - Not assessed for performance

- 12 labs reported results of a second detection method
  - 5 NRLs-*Salmonella* used qPCR
  - 5 NRLs-*Salmonella* used PCR
  - 1 NRL-*Salmonella* used mini VIDAS
  - 1 NRL-*Salmonella* incubated MKTTn at 41,5 °C

- Similar results to the prescribed method EN ISO 6579-1:2017(/Amd 1:2020)

Next

- Individual results and interim summary were sent
- Report



THANK YOU!

Questions?

EURL Salmonella