



RESULTS OF PROFICIENCY TESTS NO. 23 AND 24



Helena Höök
EURL-Campylobacter
Workshop 2019

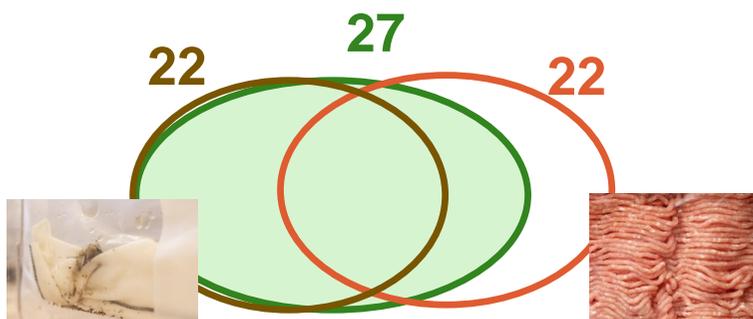




Thank you for your participation and for providing information in the questback reports!

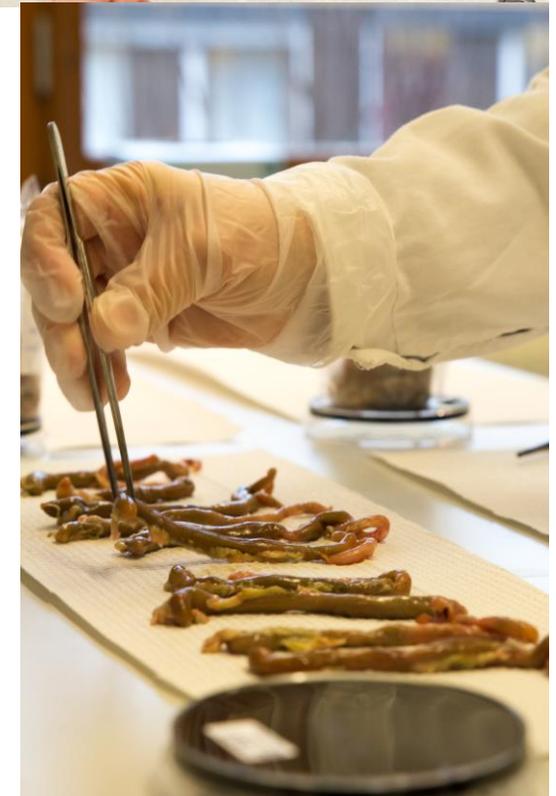
NUMBERS OF PARTICIPANTS

Year	2019	2018	2017	2016	2015	2014	2013	2012	2011
	PT 23	PT 21	PT 19	PT 17	PT 15	PT 13	PT 11	PT 9	PT 8
Enumeration	35	37	36	36	36	35	36	33	33
	PT 24	PT 22	PT 20	PT 18	PT 16	PT 14	PT 12	PT 9	PT 8
Detection & species id	33	31	34	33	32	36	34	36	34



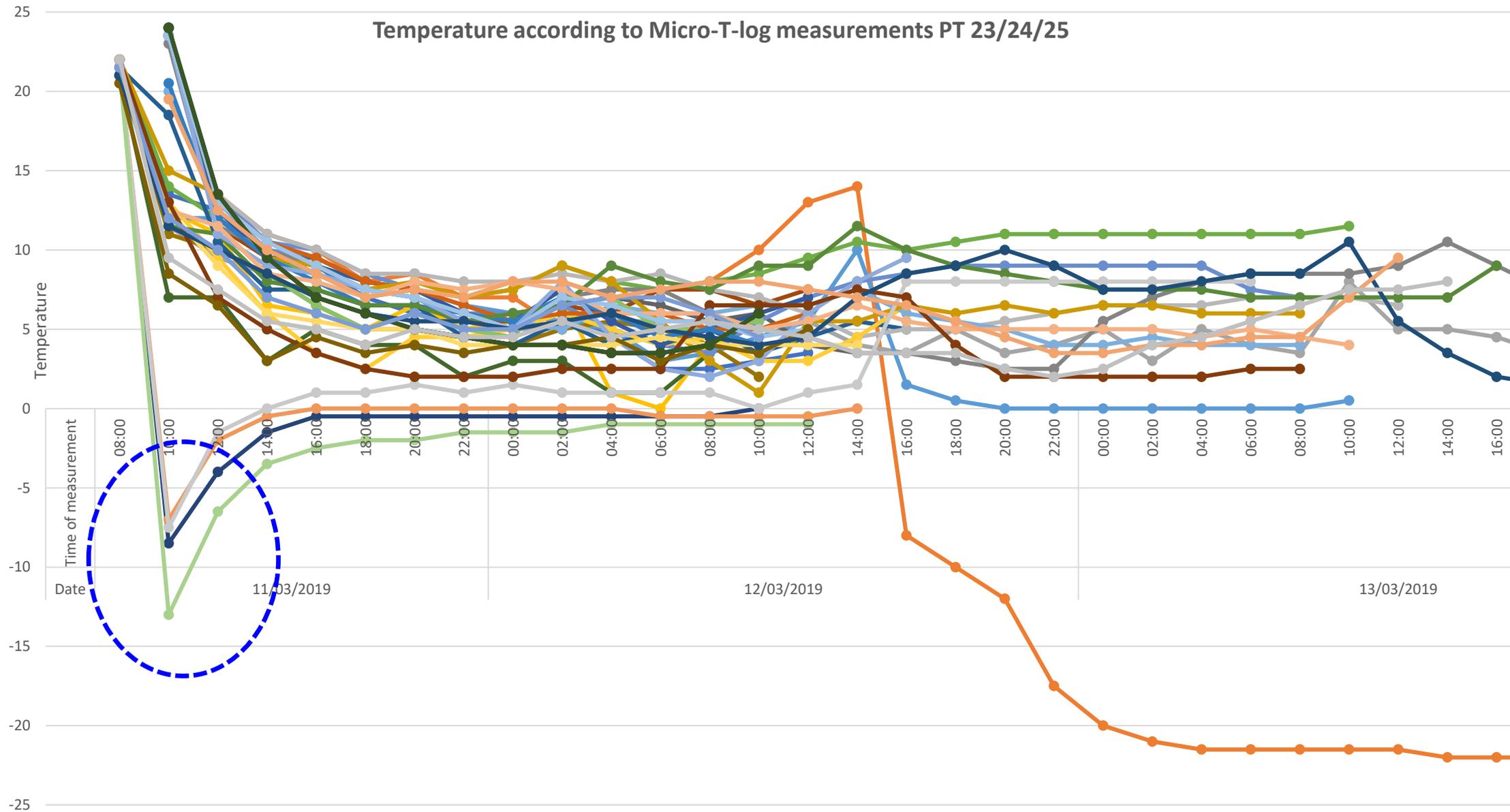
CAMPYLOBACTER-FREE MATRICES

- Chicken meat (PT 23, PT 24)
- Caecal material (PT 24)
- Litter material (PT 24)
- All from a producer with no *Campylobacter*-positive broiler flocks for several months, and a slaughterhouse with very low level of *Campylobacter*-positive flocks
- Meat, litter and caecal material tested negative for presence of *Campylobacter*



TEMPERATURE DURING TRANSPORT

Temperature according to Micro-T-log measurements PT 23/24/25



PT 23 – ENUMERATION (AND SPECIES IDENTIFICATION) IN CHICKEN MEAT



PROFICIENCY TEST NO. 23

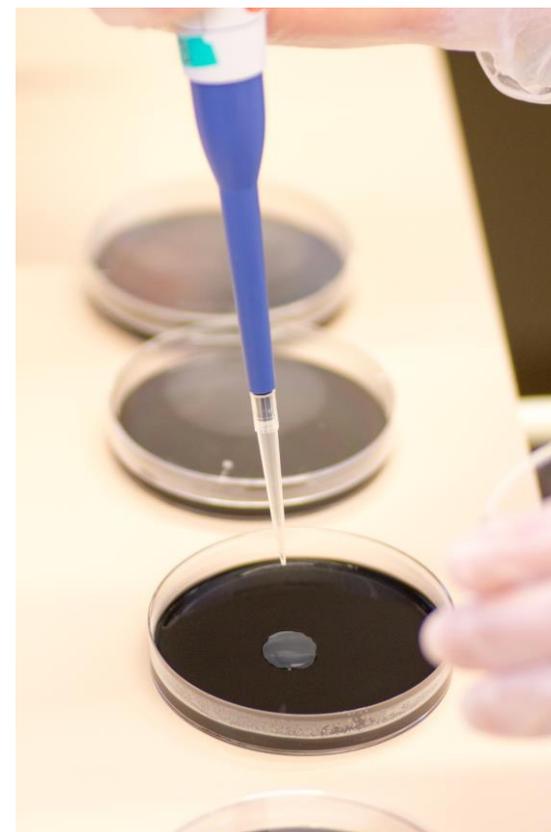
The objective was to assess the performance of the NRLs to enumerate (and voluntary species identify) *Campylobacter* in chicken meat.

- Enumeration and confirmation of *Campylobacter* spp. in chicken meat
- Species identification of *Campylobacter* (voluntary)
- Recommended method ISO 10272:2017, but other methods allowed
- Should allow enumeration of between 10 and 10⁵ cfu *Campylobacter*/g chicken meat



PT 23: CONTENTS AND PROCEDURE

- Chicken meat (about 120 g) to be divided into 10 portions of 10 g
- 10 vials with freeze-dried sample (with or without *Campylobacter*)
- Homogenise and make a initial dilution of 10^{-1}
- Follow the method(s) of choice for
 - enumeration
 - species identification (voluntary)



of *Campylobacter* spp.

DESCRIPTION OF THE 10 VIALS IN PT 23

Sample No.	Species	Level (log cfu/vial)		Batch No.
1	<i>C. jejuni</i>	3.71		SLV306
2	<i>C. lari</i>	4.82		SLV248
3	Negative			SLV289
4	<i>Escherichia coli</i>		4.46	SLV150
5	<i>C. lari</i>	4.04		SLV299
6	<i>C. jejuni</i>	3.71		SLV306
7	<i>C. jejuni</i> + <i>Escherichia coli</i>	3.50	4.00	SLV313
8	<i>C. coli</i>	5.67		SLV287
9	<i>C. jejuni</i>	4.47		SVA010
10	<i>C. coli</i>	5.67		SLV287

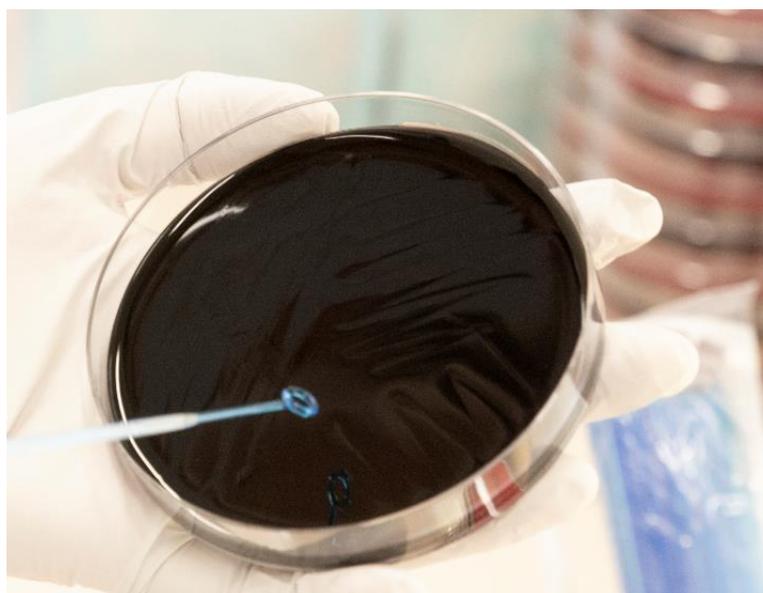
PT 23: QUALITY CONTROL

- Vials produced by the National Food Agency
- Vials tested for homogeneity and stability by the producer and in triplicates by EURL
- Enumerations with chicken meat for control of *Campylobacter* levels and homogeneity
- Tested three times, once before and twice after dispatch



PT 23: METHODS

Reported method for enumeration	No. of NRLs
ISO 10272:2017	32
NMKL 119, 3rd ed. 2007	2
Intern method	1



WHAT'S IN THE RESULTS?



- Laboratory procedures
 - Dilution
 - Spreading
 - Counting
 - Confirmation

ISO 7218

- Calculations

$$N = \frac{\sum C}{V \times 1,1 \times d} = \frac{168 + 14}{1 \times 1,1 \times 10^{-2}} = \frac{182}{0,011} =$$

- Reporting

4.2 log cfu/g *Campylobacter* spp.

- Final results

Results EURL-Campylobacter Proficiency Test Number 23 2019

Enumeration of *Campylobacter* in chicken meat

	Score	Performance
Overall enumeration	80.0%	Acceptable
Sensitivity identification (voluntary)	87.5%	Good

Country Testland

Laboratory The laboratory of food

NRL lab ID	Name of contact person (NRL)			Name of contact person (PT 23)			Date of arrival	Analysis start		
100	Test Testsson			Test Testsson			3/12/2019	3/13/2019		
	Sample 1.	Sample 2.	Sample 3.	Sample 4.	Sample 5.	Sample 6.	Sample 7.	Sample 8.	Sample 9.	Sample 10.
Contents	<i>Campylobacter jejuni</i>	<i>Campylobacter lari</i>	Negative	<i>Escherichia coli</i>	<i>Campylobacter lari</i>	<i>Campylobacter jejuni</i>	<i>Campylobacter jejuni</i> <i>Escherichia coli</i>	<i>Campylobacter coli</i>	<i>Campylobacter jejuni</i>	<i>Campylobacter coli</i>
Batch No.	SLV306	SLV248	SLV289	SLV150	SLV299	SLV306	SLV313	SLV287	SLV305	SLV287

Enumeration of *Campylobacter* spp. (mandatory)

value below median value -3σ MAD / z-score below -3	value between median value -2σ MAD and -3σ MAD / z-score between -2 and -3
value above median value $+3\sigma$ MAD / z-score above 3	value between median value $+2\sigma$ MAD and $+3\sigma$ MAD / z-score between 2 and 3
false positive	

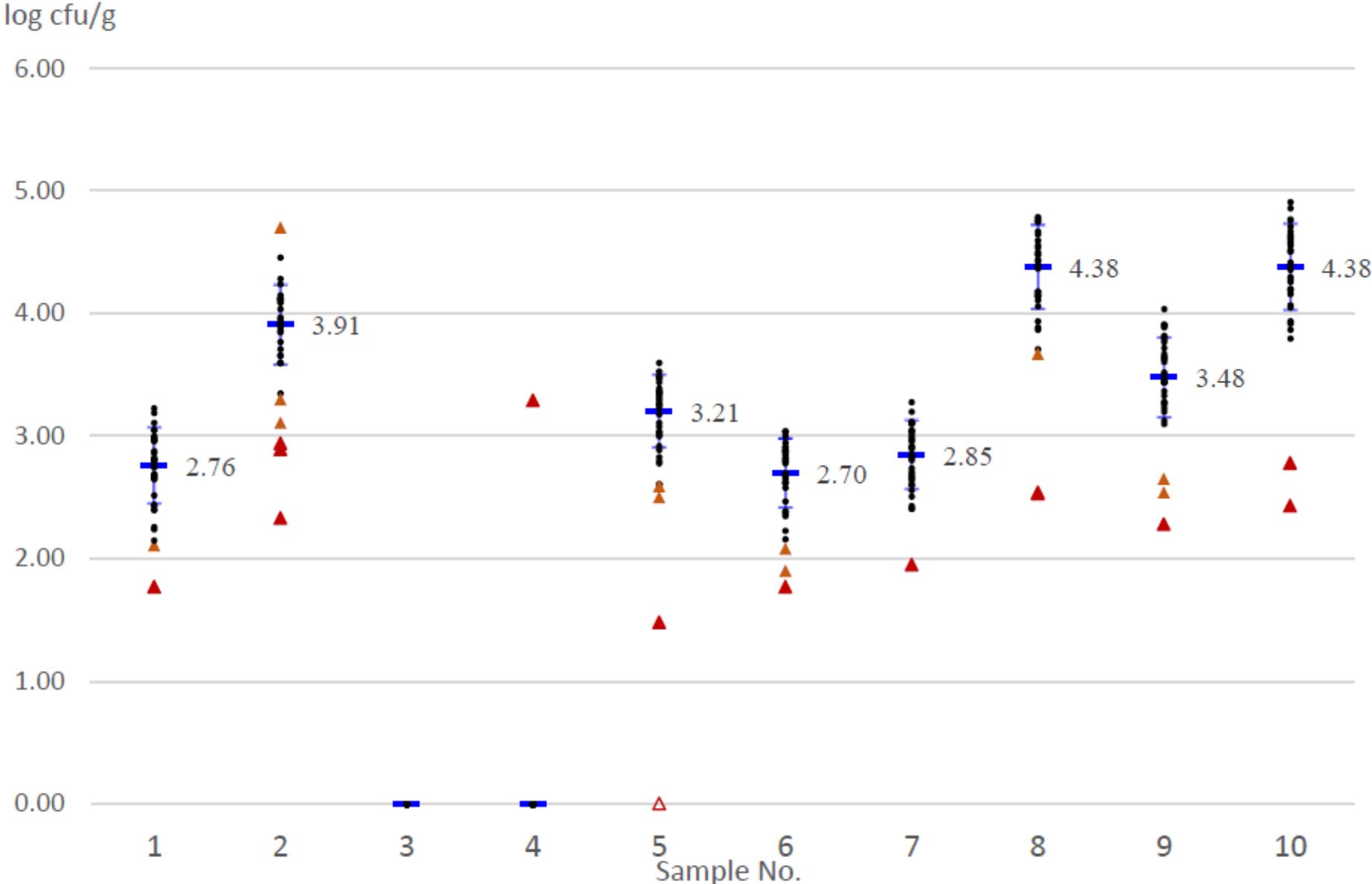
Lab's results enumeration (log cfu/g)	2.75	4.45	<1.00	<1.00	2.56	2.58	2.69	2.68	4.43	4.56
Results as reported	2.75	4.45	0	<1.0	2.56	2.58	2.69	2.68	4.43	4.56
Score (points)	2	2	2	2	1	2	2	0	1	2
Z-score	-0.03	1.66	-	-	-2.18	-0.43	-0.57	-4.99	2.91	0.51
Median	2.76	3.91	<1.00	<1.00	3.21	2.70	2.85	4.38	3.48	4.38
MADe	0.21	0.22	-	-	0.20	0.19	0.19	0.23	0.22	0.24
σ MADe	0.31	0.33	-	-	0.30	0.28	0.28	0.34	0.33	0.35
$+2\sigma$ MADe	3.39	4.57	-	-	3.80	3.27	3.42	5.07	4.14	5.09
-2σ MADe	2.13	3.25	-	-	2.61	2.13	2.28	3.69	2.82	3.67
$+3\sigma$ MADe	3.70	4.89	-	-	4.10	3.55	3.70	5.41	4.46	5.44
-3σ MADe	1.82	2.93	-	-	2.31	1.80	2.00	3.30	2.50	3.32

Species identification of *Campylobacter* spp. (voluntary)

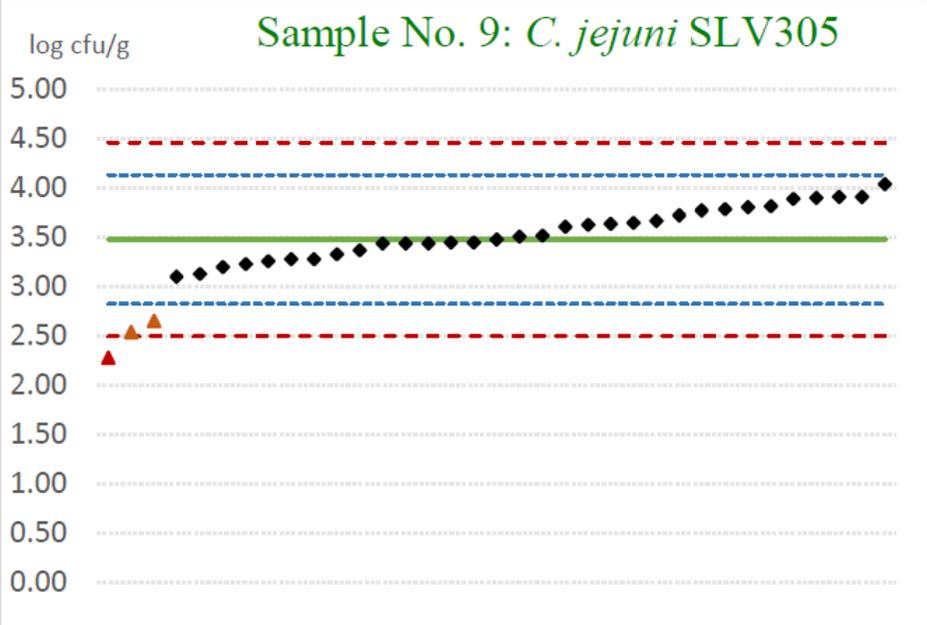
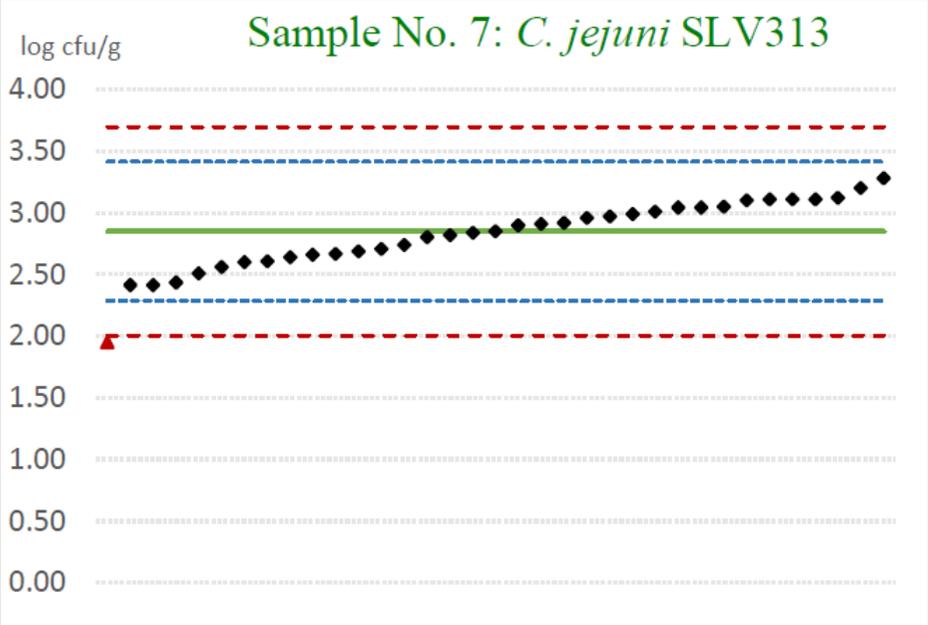
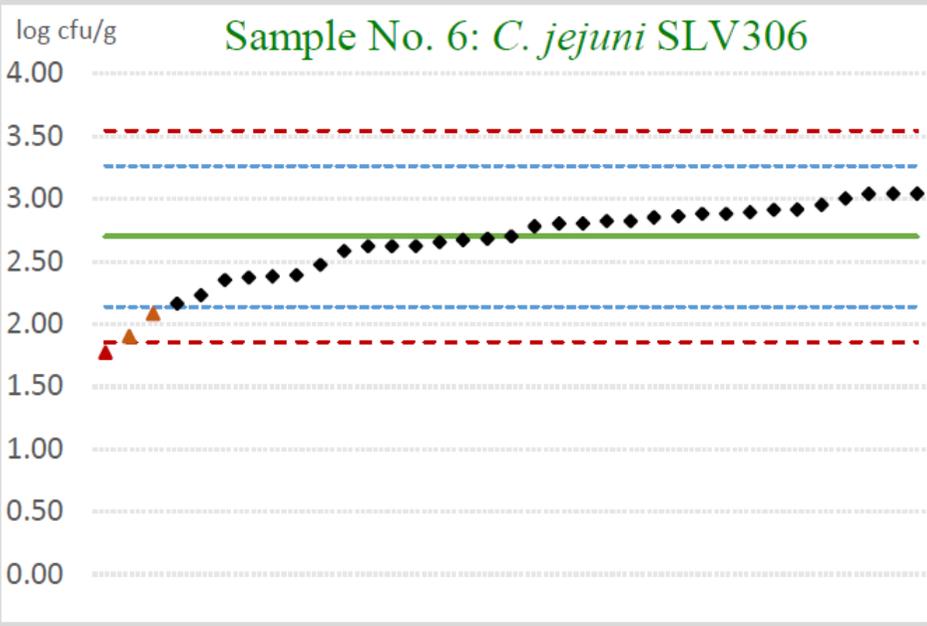
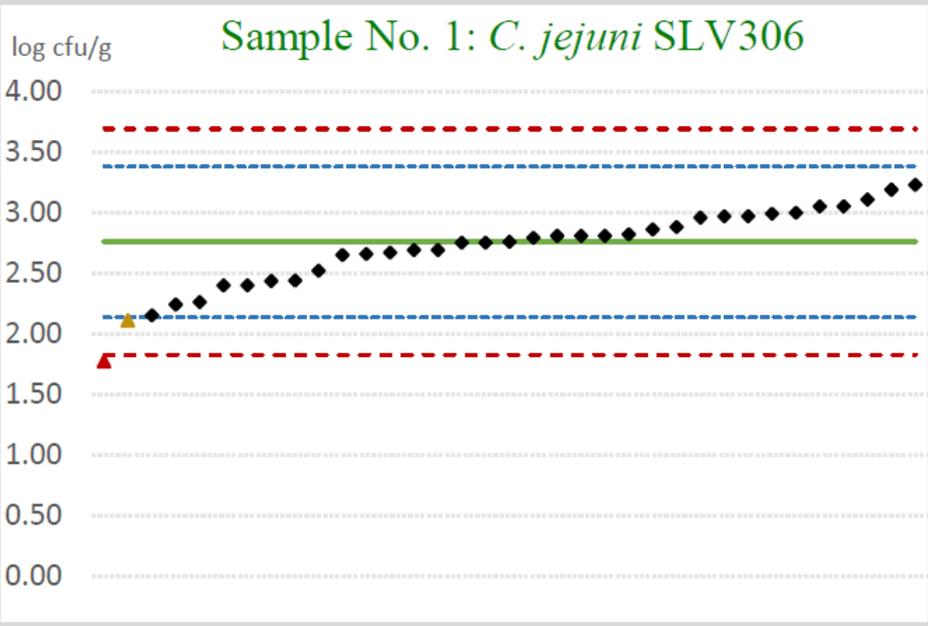
incorrect/no species identification	false positive
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Lab's results species identification	<i>Campylobacter jejuni</i>	<i>Campylobacter lari</i>	No growth at all	Growth of other, not <i>Campylobacter</i>	<i>Campylobacter lari</i>	<i>Campylobacter coli</i>	<i>Campylobacter jejuni</i>	<i>Campylobacter coli</i>	<i>Campylobacter jejuni</i>	<i>Campylobacter coli</i>
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PT 23: RESULTS OF ENUMERATION



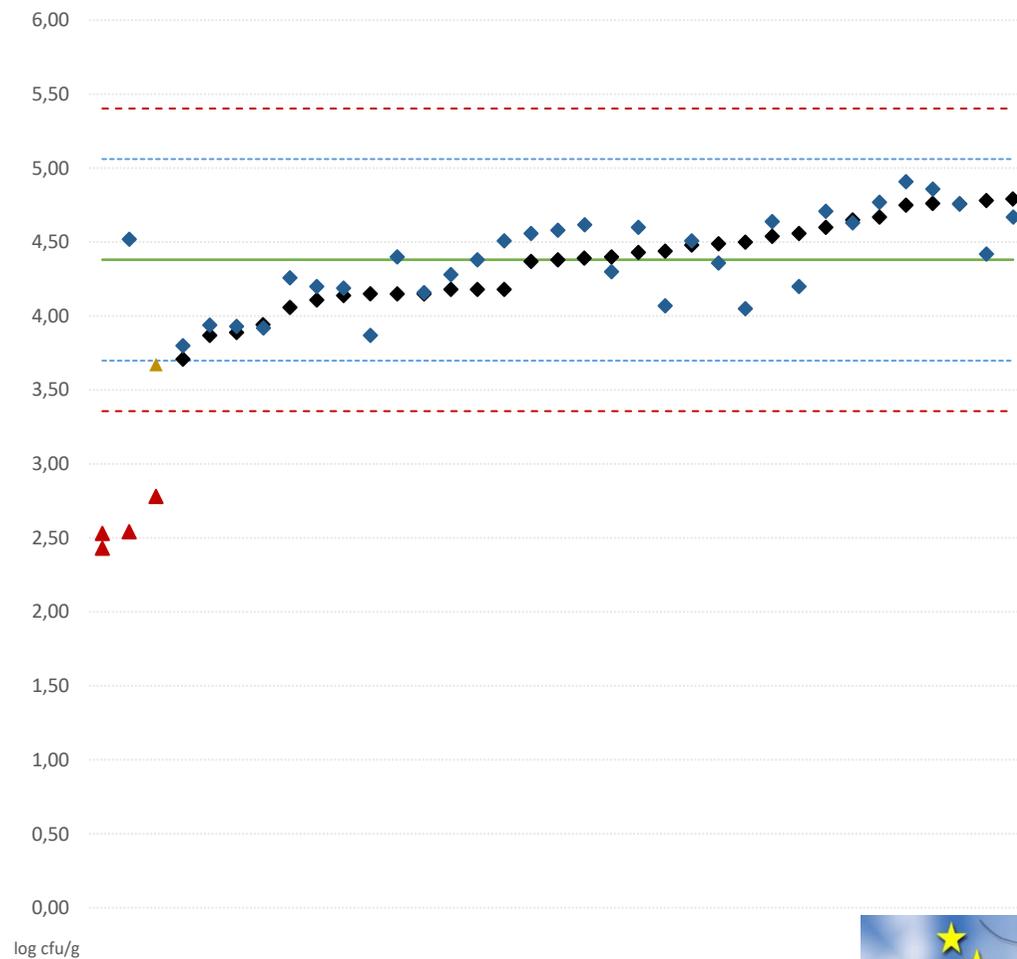
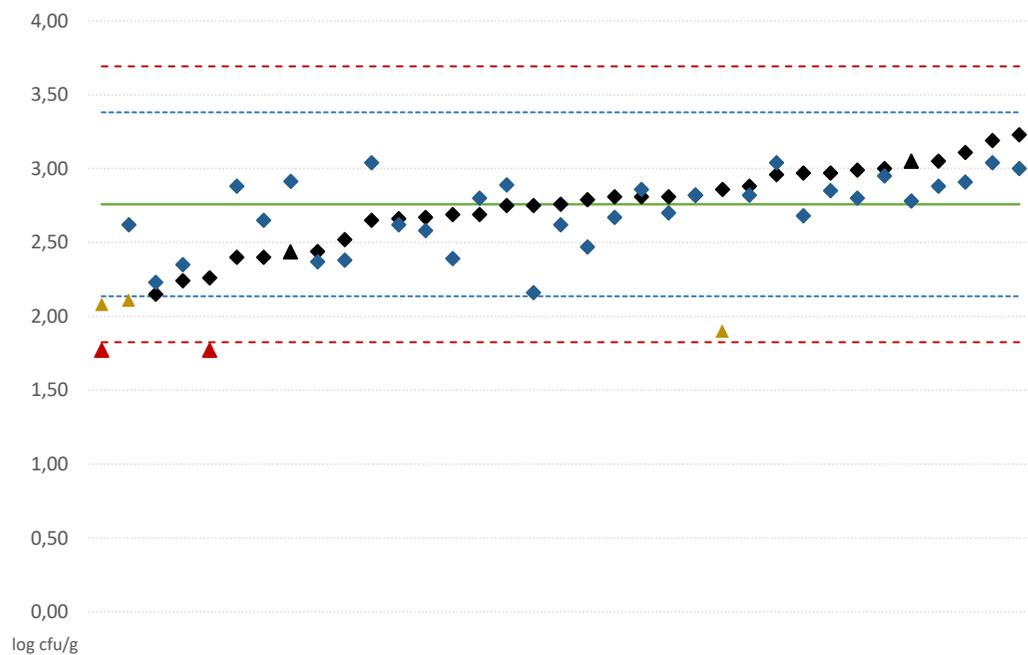
PT 23: RESULTS OF ENUMERATION



PT 23: RESULTS OF ENUMERATION

Sample No. 8 and 10: *C. coli* SLV287

Sample No. 1 and 6: *C. jejuni* SLV306

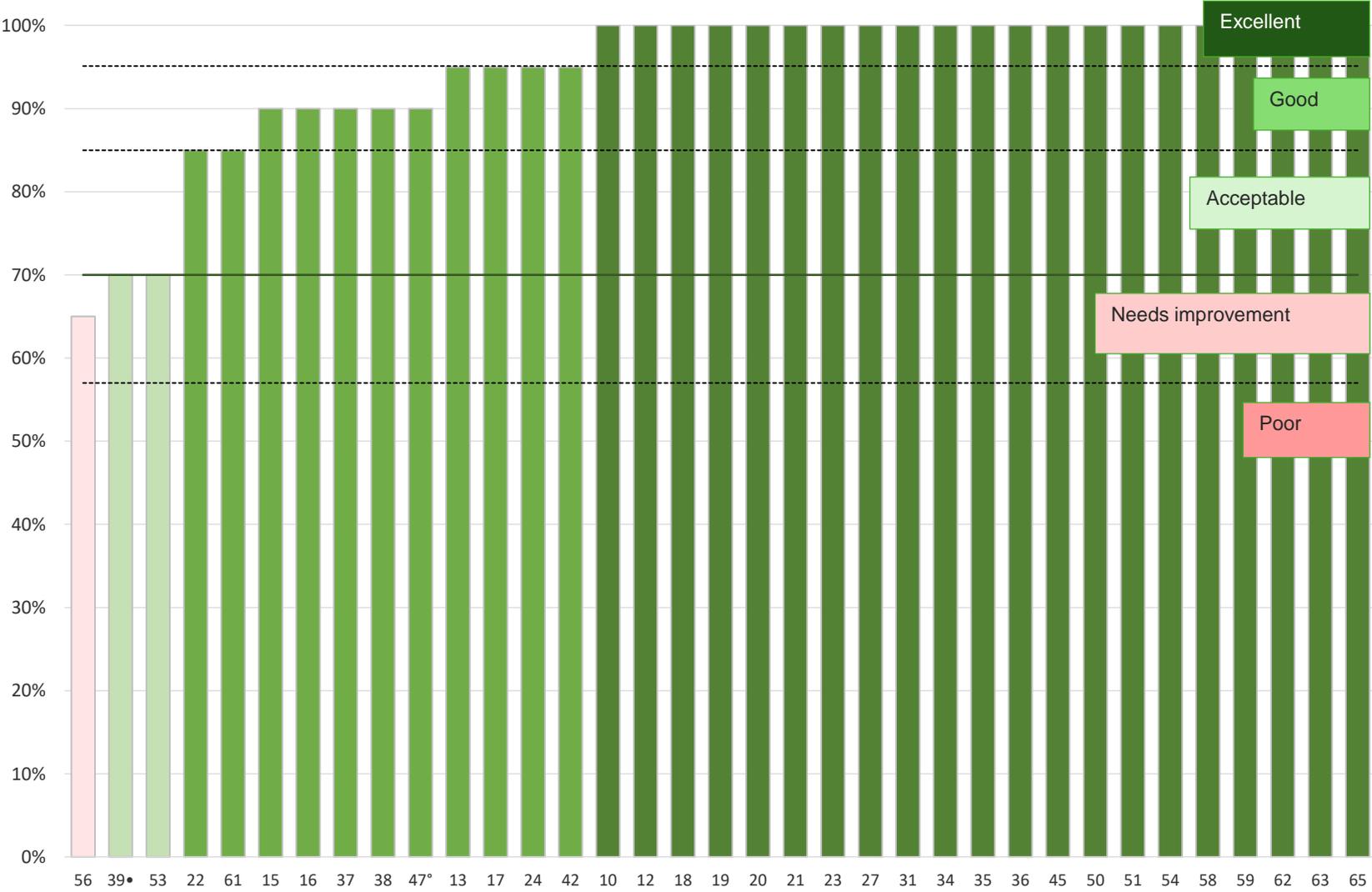


HOW WAS PERFORMANCE CALCULATED?

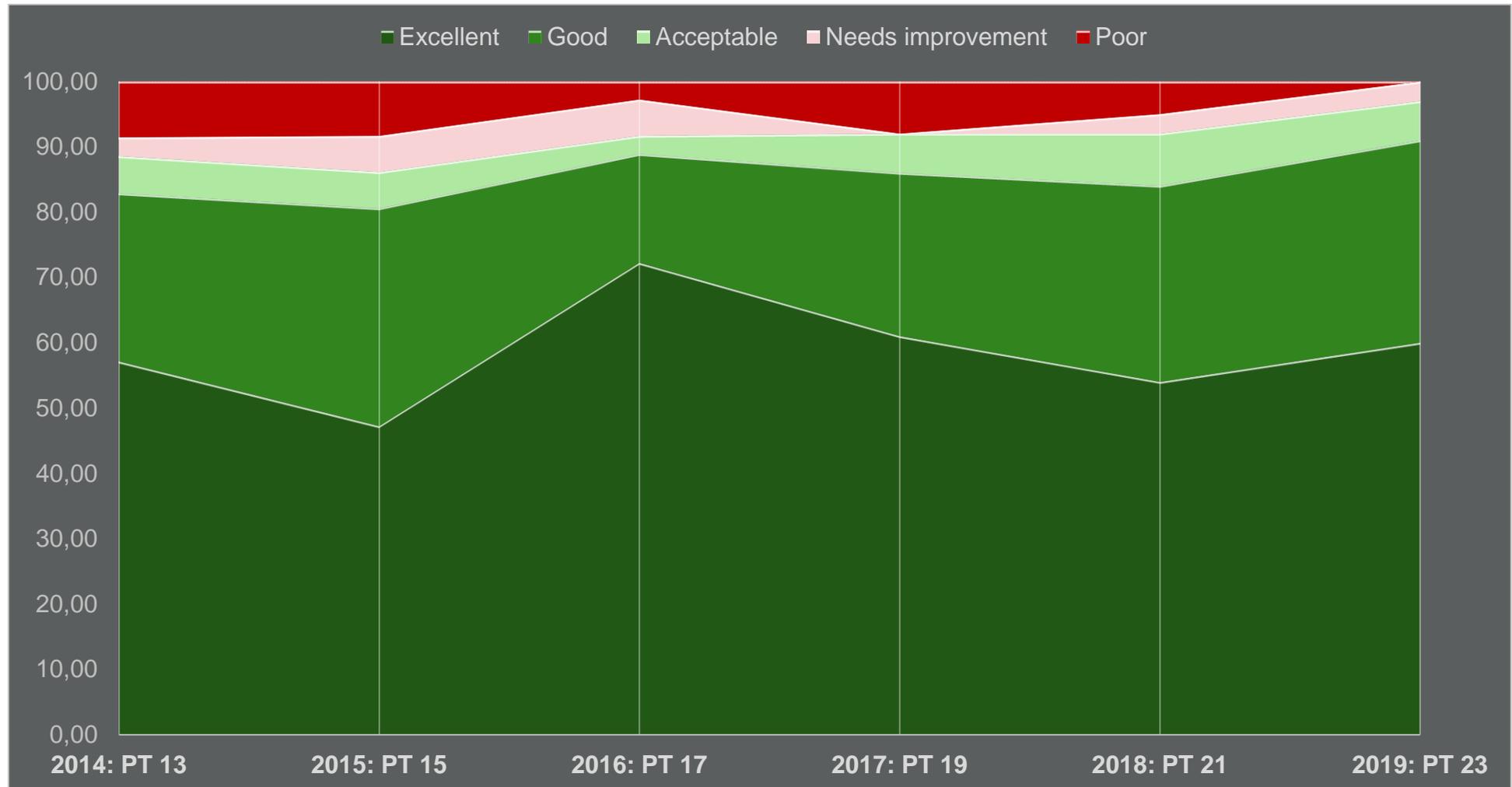
- The Median Absolute Deviation (MADe) to calculate performance
- $\sigma\text{MADe} = \text{MADe} \times 1.4826$
- *Campylobacter*-containing samples
 - Results within participants' median $\pm 2\sigma\text{MADe} = 2$ points
 - Results between $\pm 2\sigma\text{MADe}$ and $\pm 3\sigma\text{MADe} = 1$ point
 - Results outside $\pm 3\sigma\text{MADe} = 0$ points
- *Campylobacter*-negative samples
 - No *Campylobacter* reported = 2 points
 - False positive result = 0 points
- The maximum score (2 points for each sample) was 20 points
- Calculate the score for each participant

Grade	Scoring limits	
Excellent	20	95.1–100%
Good	17–19	85.0–95.0%
Acceptable	14–16	70.0–84.9%
Needs improvement	12–13	57.0–69.9%
Poor	<12	<57.0%

PERFORMANCE PT 23



PERFORMANCE IN ENUMERATION OVER TIME



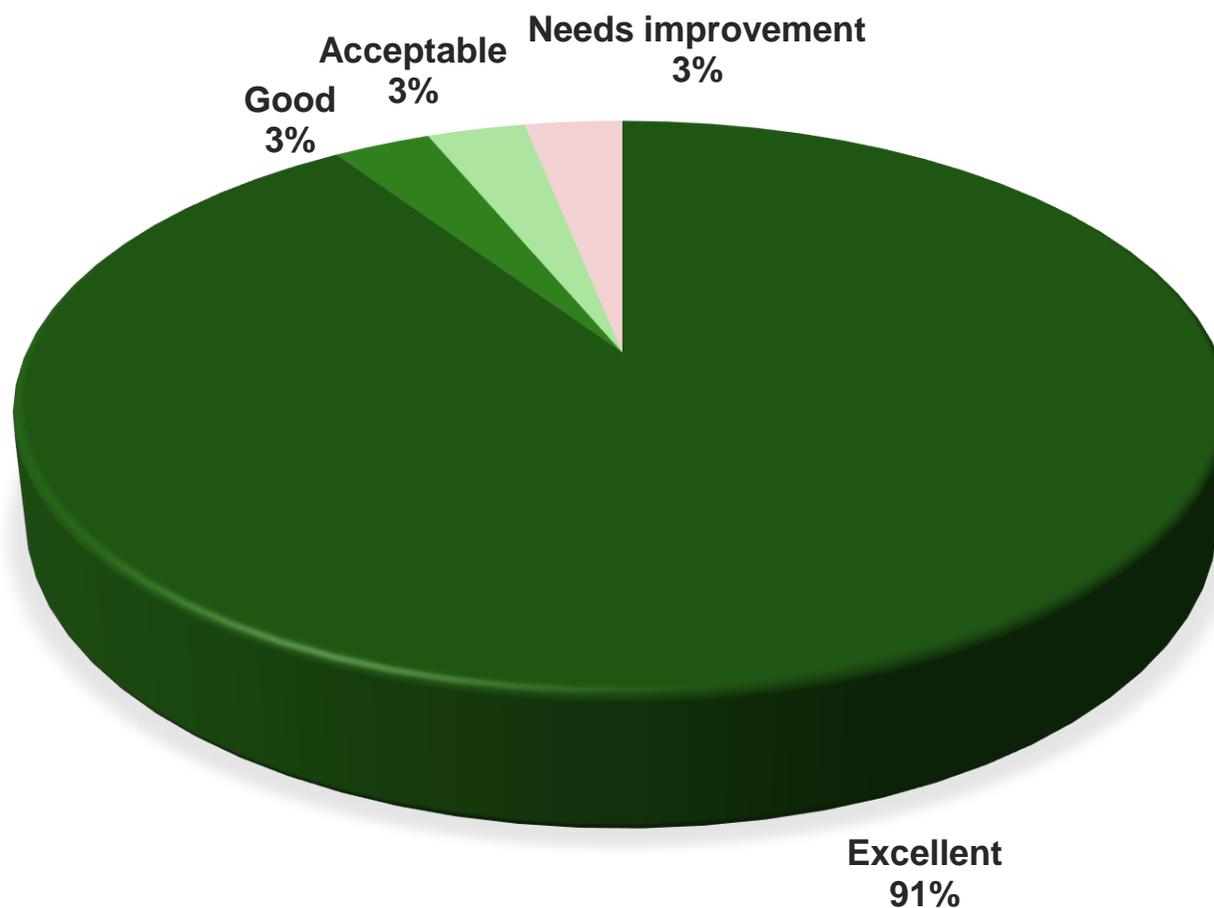
PT 23: PERFORMANCE IN RELATION TO START OF ANALYSIS

Day	Number of NRLs	Performance				
		Excellent	Good	Acceptable	Needs improvement	Poor
12 th of March	2	1	1			
13 th of March	11	8	3			
14 th of March	3	2	1			
17 th of March	1		1			
18 th of March	8	5	2	1		
19 th of March	3	1	1	1		
20 th of March	1				1	
21 st of March	1	1				
25 st of March	4	2	2			
26 th of March	1	1				

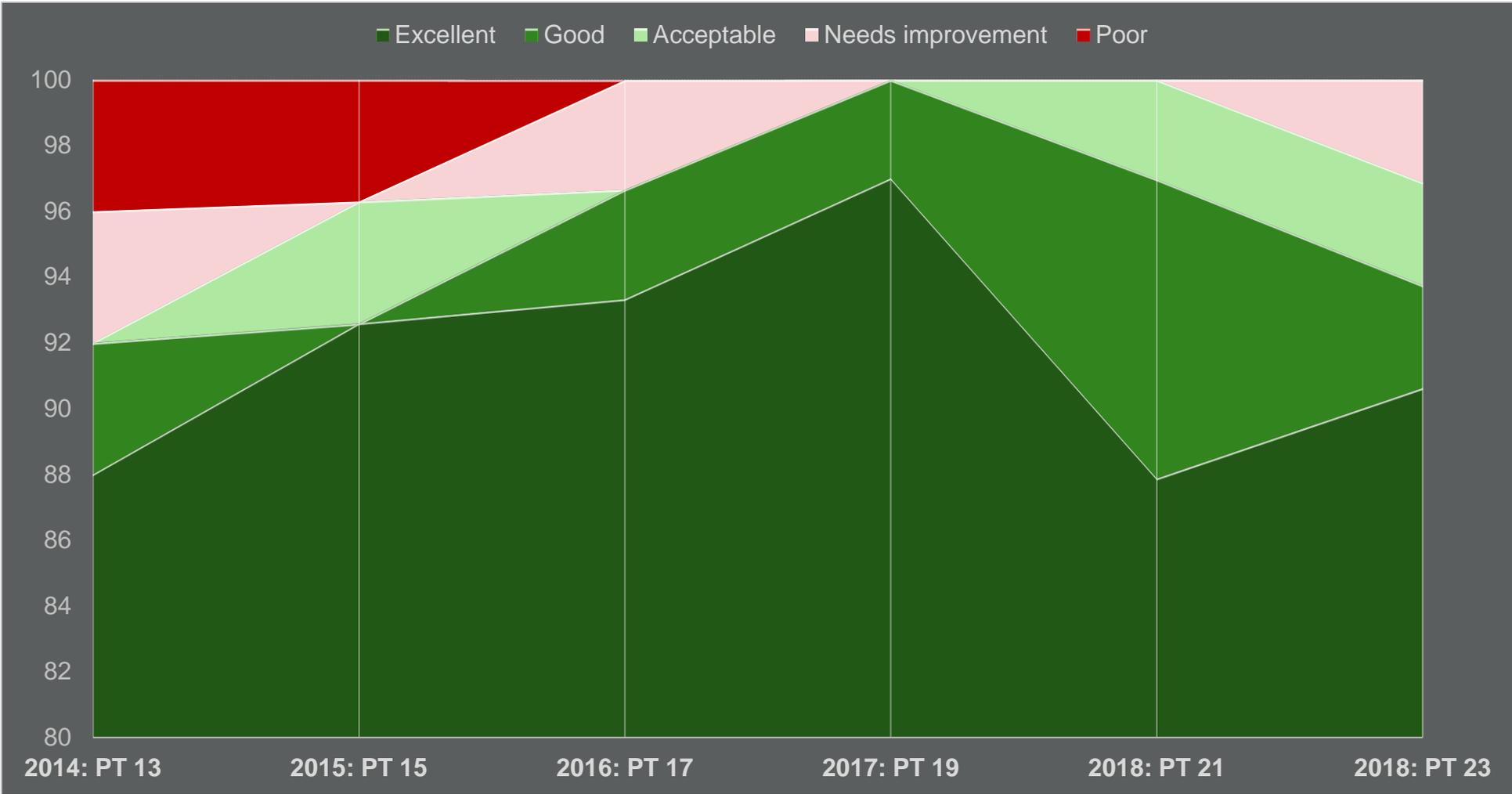
PT 23: SPECIES IDENTIFICATION (VOLUNTARY)

Content of sample (vial)	<i>C. jejuni</i>	<i>C. coli</i>	<i>C. lari</i>	Camp spp.	Other / No growth
1. <i>C. jejuni</i>	32				
2. <i>C. lari</i>			32		
3. Negative					32
4. <i>E. coli</i>					32
5. <i>C. lari</i>			31	1	
6. <i>C. jejuni</i>	32				
7. <i>C. jejuni</i> + <i>E. coli</i>	30		1	1	
8. <i>C. coli</i>	1	31			
9. <i>C. jejuni</i>	30	1		1	
10. <i>C. coli</i>		32			

PERFORMANCE PT 23: SENSITIVITY IN SPECIES IDENTIFICATION (VOLUNTARY)



PERFORMANCE IN IDENTIFICATION (SE) OVER TIME



PT 24 – DETECTION AND SPECIES IDENTIFICATION OF *CAMPYLOBACTER*



PROFICIENCY TEST NO. 24

The objective was to assess the performance of the NRLs to detect and identify *Campylobacter* species in minced chicken meat and/or boot sock samples.

- Detection of *Campylobacter* spp. in minced chicken meat / boot sock samples
- Species identification of *Campylobacter*
- Three sets:
 - 10 core samples of **minced chicken meat**
 - 10 core **sock samples** mimicking samples taken in a chicken house with birds kept indoors
 - 4 **educational** sock samples (not included in the performance evaluation) mimicking samples taken in a chicken pen with birds kept outdoors
- Recommended method ISO 10272:2017, but other methods allowed
- Sample preparation boot socks: according to routine procedure in the laboratory or instructions sent out (for procedures A, B or C in ISO 10272:2017)

PT 24: CONTENTS AND PROCEDURE: MINCED CHICKEN MEAT

- Plastic bag with minced chicken meat (about 120 g)
- 10 freeze-dried vials (with or without *Campylobacter*)
- Homogenise the meat (like a laboratory sample) before divided into 10 portions of 10 g
- From here on, treat each 10 g test portion as a separate sample
- Mix each vial with 10 g minced chicken meat
- Follow the method(s) of choice for
 - detection
 - species identification

of *Campylobacter* spp.



PT 24: CONTENTS AND PROCEDURE: SOCK SAMPLES

- 10 numbered boot sock samples in plastic bags
- 10 freeze-dried vials (with or without *Campylobacter*)
- Mix each vial with enrichment broth or other liquid and pour into the bag with sock sample
- Follow the method(s) of choice for
 - detection
 - species identificationof *Campylobacter* spp.



PT 24: CORE SAMPLES MINCED CHICKEN MEAT

Sample No.	Content in vial	Batch No.	Level	log cfu/vial
11	<i>Campylobacter lari</i>	SVA016	High	4.38
12	<i>Campylobacter jejuni</i>	SVA021	High	4.28
13	<i>Campylobacter coli</i>	SVA023	Low	2.93
14	Negative	SLV289		
15	Negative	SLV289		
16	<i>Campylobacter coli</i>	SVA022	High	3.45
17	<i>Campylobacter lari</i>	SVA017	Low	3.27
18	<i>Campylobacter jejuni</i>	SVA027	Low	2.02
19	<i>Campylobacter jejuni</i>	SVA025	Low	3.20
20	<i>Campylobacter jejuni</i>	SVA025	Low	3.20

Candida albicans was added as background flora in the chicken meat



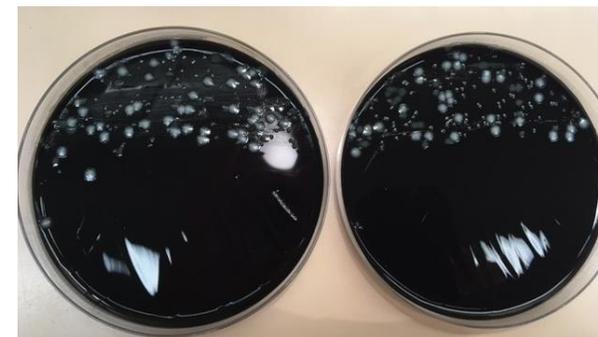
PT 24: BOOT SOCK SAMPLES

Sample No.	Content in vial	Batch No.	Level	log cfu/vial	Sock
21	<i>Campylobacter jejuni</i>	SVA025	Low	3.20	<i>E. coli</i>
22	Negative	SLV289			<i>E. coli</i>
23	<i>Campylobacter jejuni</i>	SVA027	Low	2.02	
24	<i>Campylobacter jejuni</i>	SVA021	High	4.28	<i>E. coli</i>
25	<i>Campylobacter lari</i>	SVA017	Low	3.27	
26	<i>Campylobacter jejuni</i>	SVA025	Low	3.20	
27	<i>Campylobacter lari</i>	SVA016	High	4.38	<i>E. coli</i>
28	<i>Campylobacter coli</i>	SVA022	High	3.45	
29	Negative	SLV289			
30	<i>Campylobacter coli</i>	SVA023	Low	2.93	<i>E. coli</i>

EDUCATIONAL SAMPLES

Sample No.	Content in vial	Batch No.	Level (log cfu/vial)
31	<i>Campylobacter lanienae</i>	SVA019	3.75
32	<i>Campylobacter helveticus</i>	SVA026	6.10
33	<i>Campylobacter upsaliensis</i>	SVA018	4.47
34	<i>Campylobacter lari</i> + <i>Campylobacter jejuni</i>	SVA015	4.48 (in total)

PT 24: QUALITY CONTROL



- Vials produced by EURL or the National Food Agency (negatives)
- Tested for homogeneity and stability by the producer
- Non-*Campylobacter* (*E. coli*, *Candida albicans*) strains were tested for use as live cultures
- Vials together with matrix were analysed according to ISO 10272-1:2017:
 - Chicken meat: procedure A (Bolton)
 - Sock samples: procedure A (Bolton), B (Preston) and C (direct streaking)
- Tested three times, once before and twice after dispatch



PT 24: PREPARATION OF THE MATRIX: MINCED CHICKEN MEAT

- *Campylobacter*-free chicken meat was grinded and refreezed
- Minced chicken meat was thawed at 4 °C
- An overnight culture with *Candida albicans* was prepared
- On the day of dispatch, the minced chicken meat was mixed with the overnight culture, homogenised and divided in 120 g portions (one for each participant)



PT 24: PREPARATION OF THE MATRIX: BOOT SOCK SAMPLES

- An overnight culture with *E. coli* was prepared
- *Campylobacter*-free caeca were cut and placed in a stomacher bag and mixed with Cary Blair transport medium
- For samples with background, the overnight culture was mixed with the caecum suspension
- For samples without background, serum broth of the same volume was added to the caecum suspension
- 20 ml of the suspension (with or without background) were added to a plastic bag with a boot sock, one for each sample
- The sock samples were stored at 4 °C over the weekend



PT 24: TIME TO ARRIVAL & START OF ANALYSIS

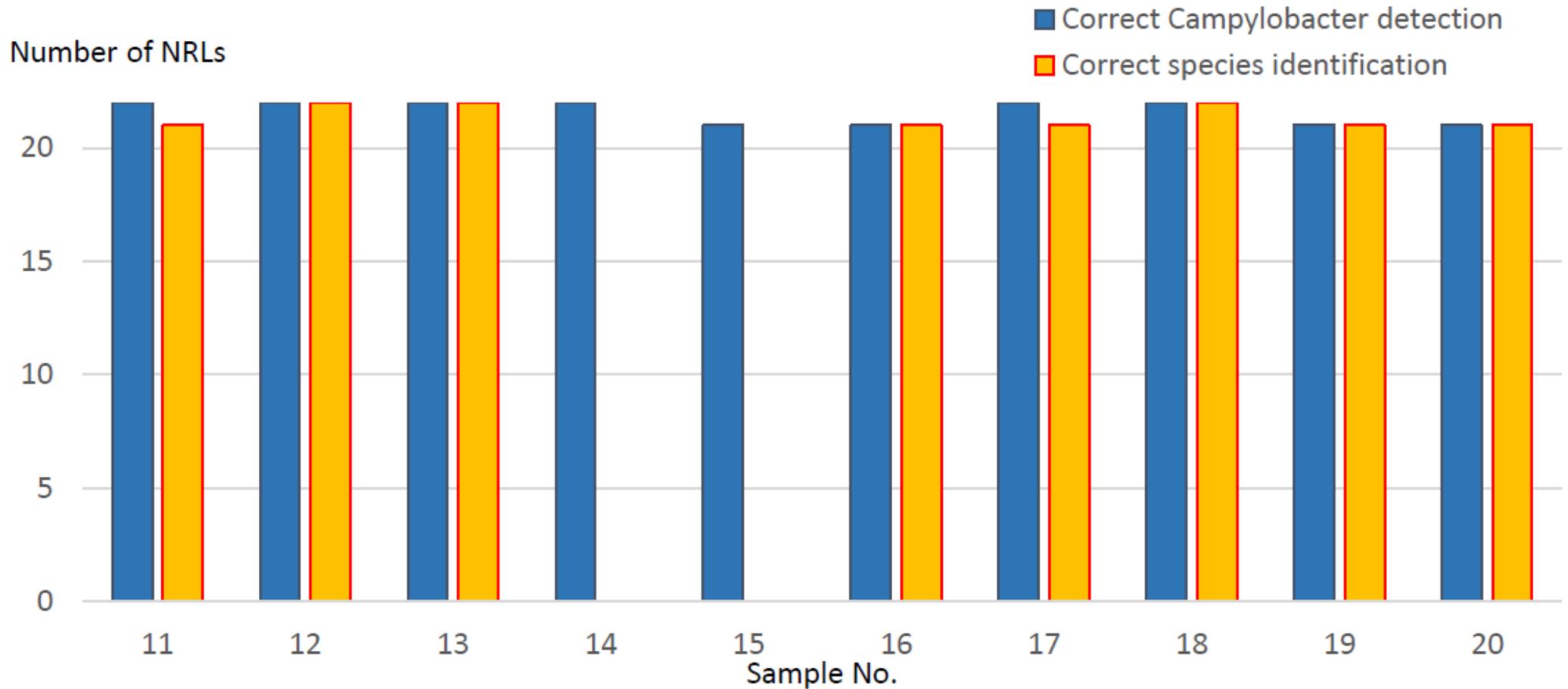
March																				
11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
<p>Dispatch from the EURL</p> <p>Arrival</p> <p>Analysis (start)</p> <p>Analysis of the samples included in PT 24 should be started as soon as possible after arrival and no later than the 15th of March 2019. Until analysis, minced chicken meat and sock samples should be stored at cold temperature (between 1°C and 8°C).</p>																				

PT 24: METHODS AND PROCEDURES

Test	ISO 10272-1:2017	Other methods	Enrich-ment	Bolton (A)	Preston (B)	Other	Direct (C)
Meat	19	3	22	16	7	1	2
Socks	21	1	20	10	10		5
Educ	23	4	24	15	10	1	9



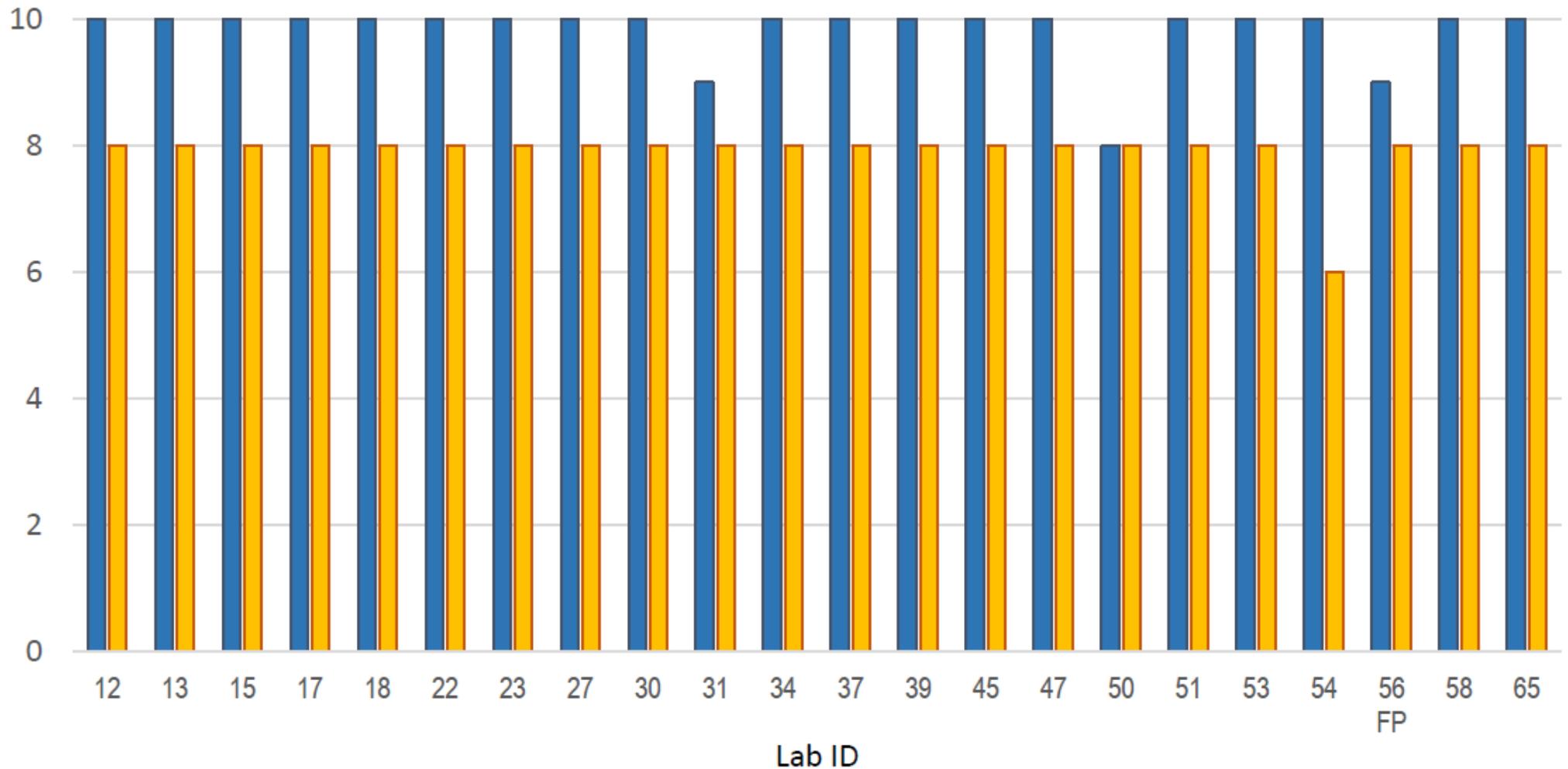
PT 24: CORRECT REPORTED RESULTS PER SAMPLE (MINCED CHICKEN MEAT)



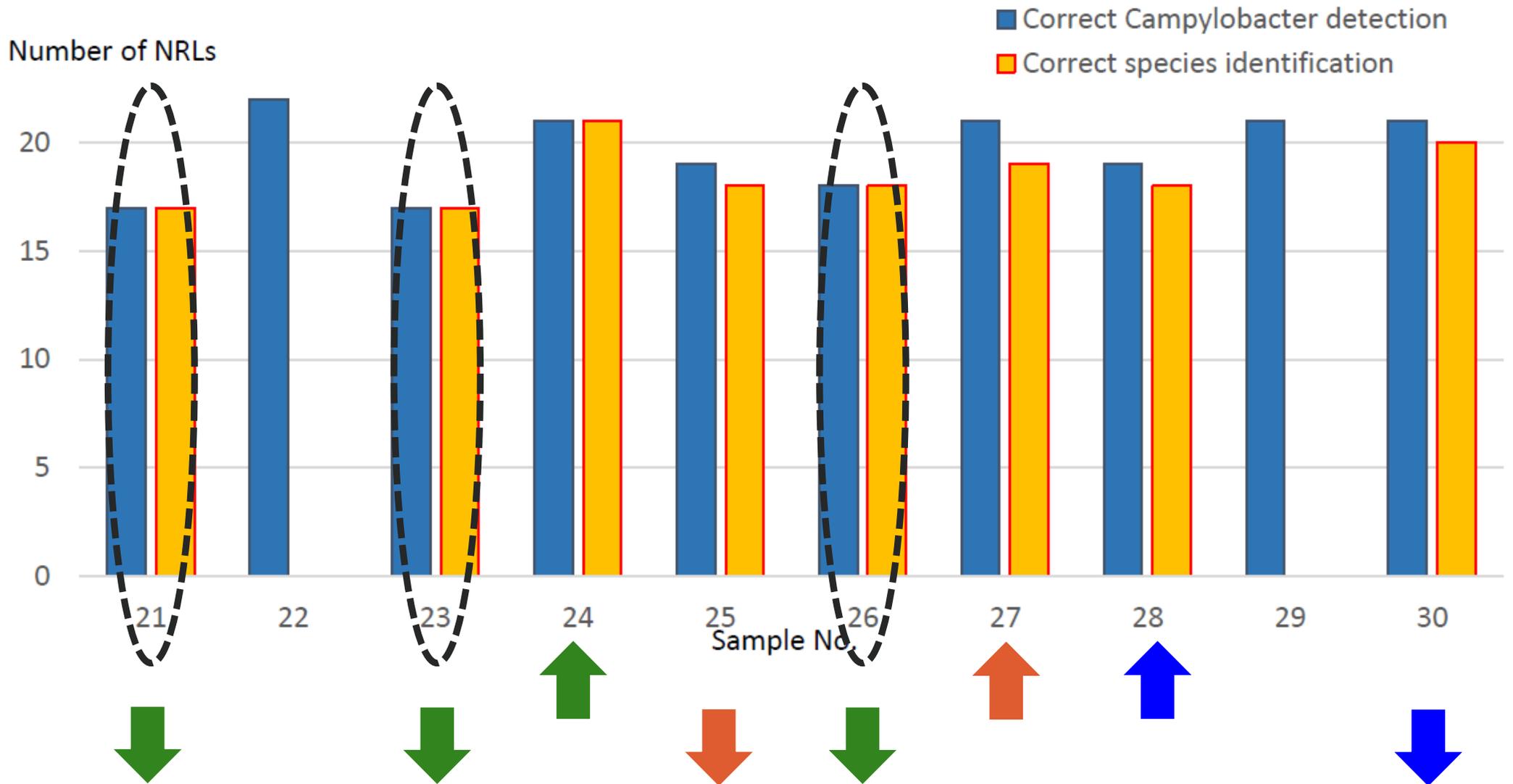
PT 24: CORRECT REPORTED RESULTS PER LAB (MINCED CHICKEN MEAT)

Number of correct reported samples

■ Correct Campylobacter detection ■ Correct species identification



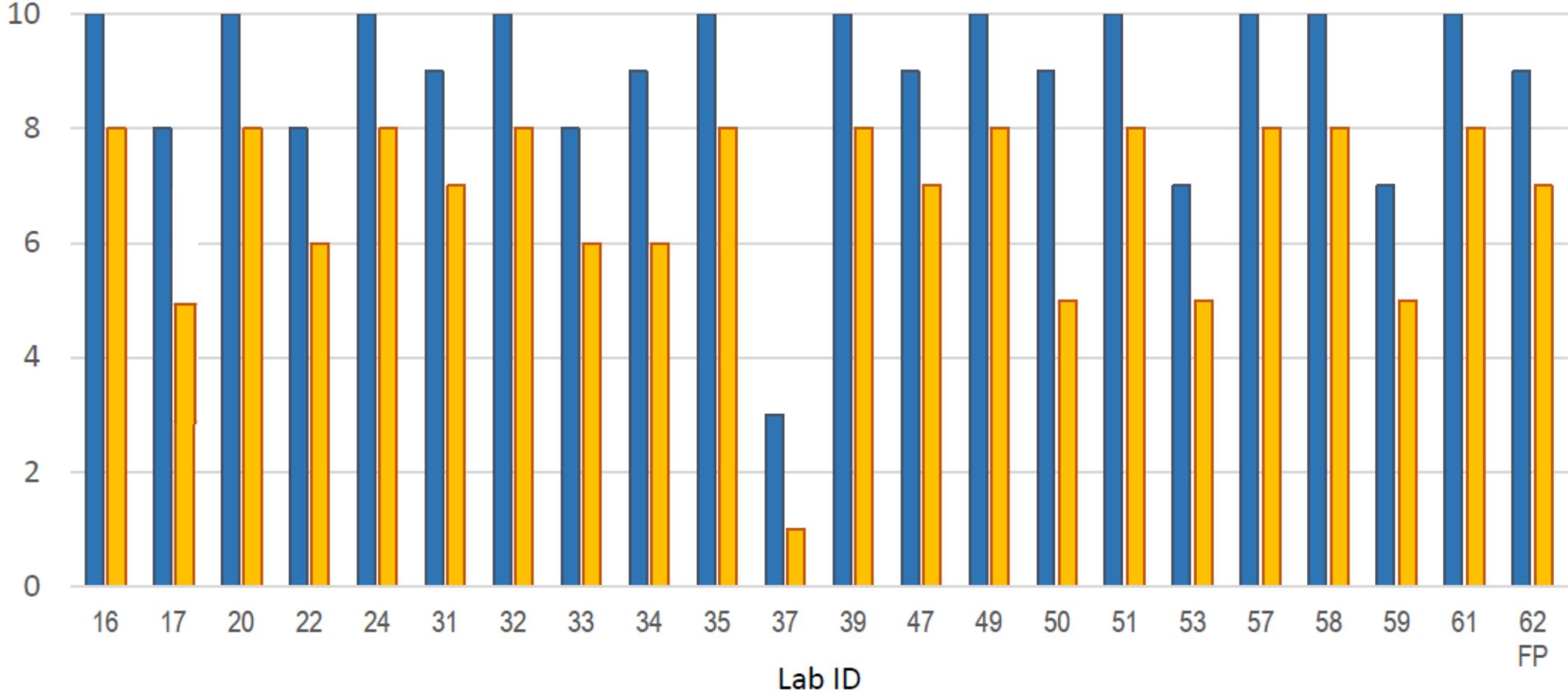
PT 24: CORRECT REPORTED RESULTS PER SAMPLE (SOCK SAMPLES)



PT 24: CORRECT REPORTED RESULTS PER LAB (SOCK SAMPLES)

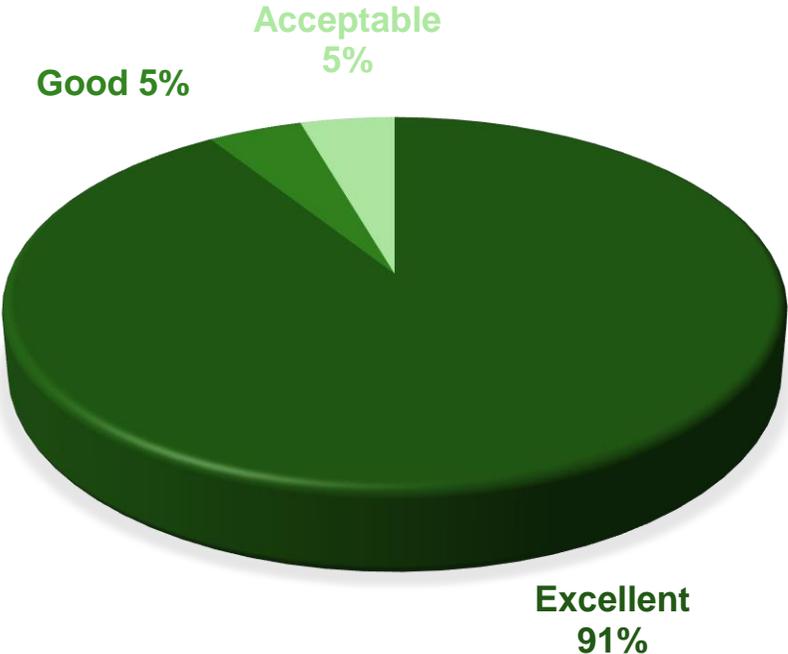
Number of correct reported samples

■ Correct Campylobacter detection ■ Correct species identification

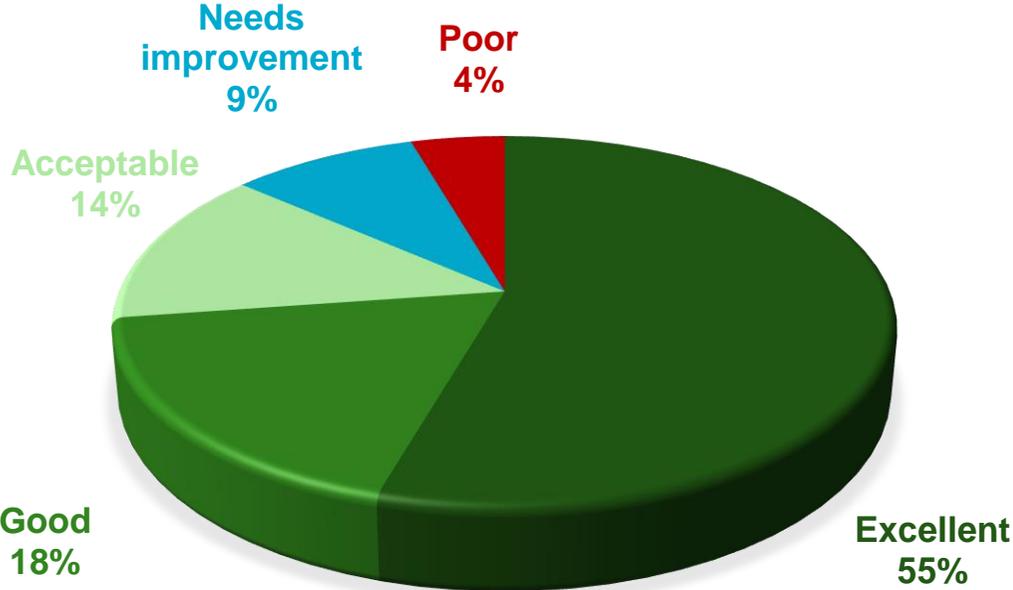


PT 24: PERFORMANCE – SENSITIVITY (SE) IN DETECTION OF *CAMPYLOBACTER*

MINCED CHICKEN MEAT

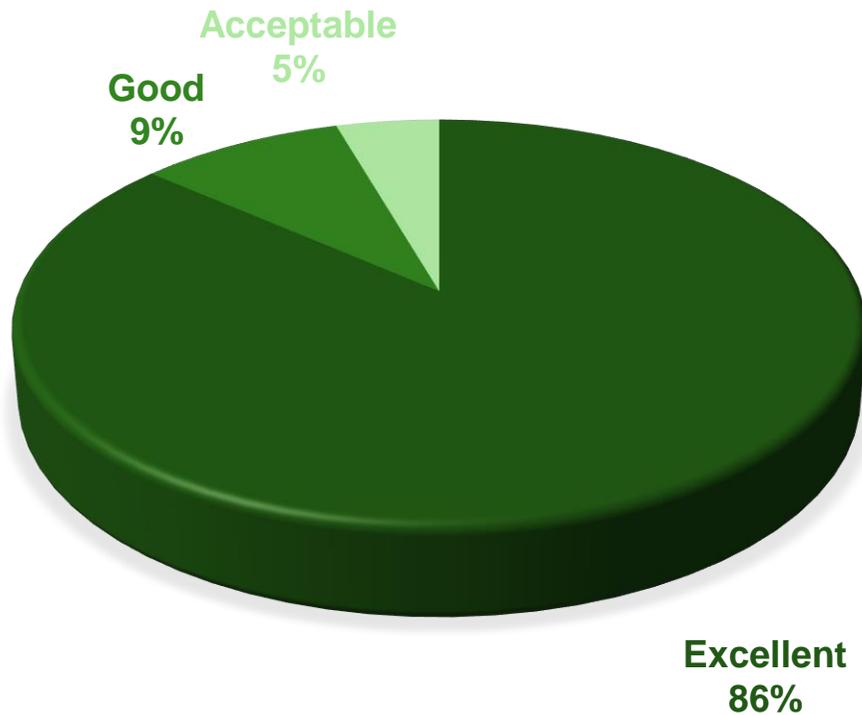


BOOT SOCK SAMPLES

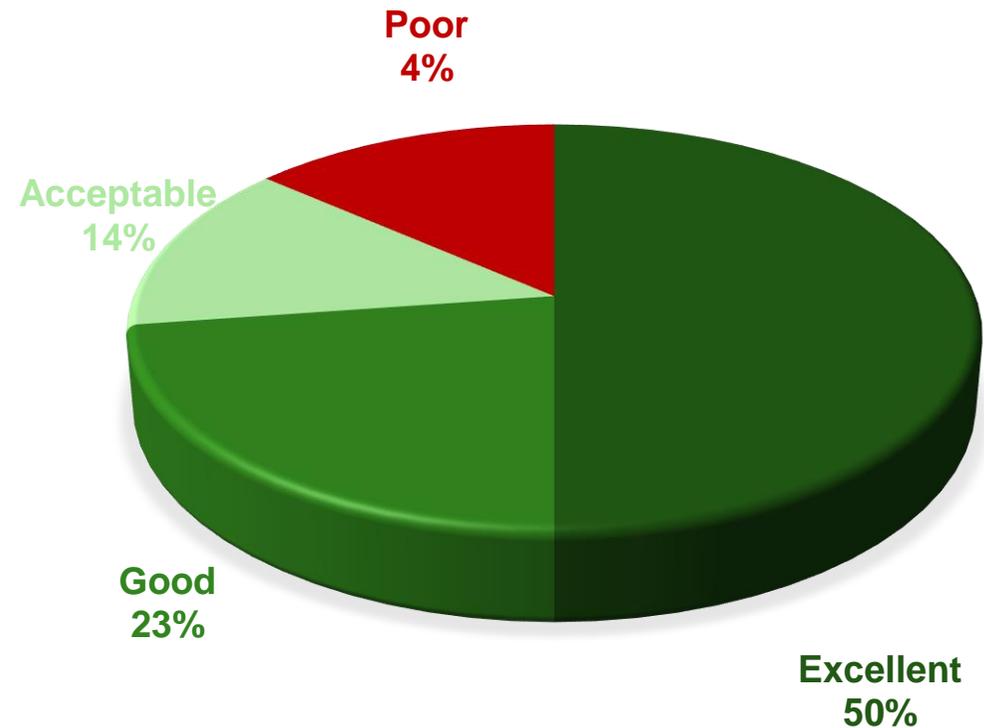


PT 24: ACCURACY IN DETECTING POSITIVE AND NEGATIVE *CAMPYLOBACTER* SAMPLES

MINCED CHICKEN MEAT



BOOT SOCK SAMPLES



PT 24: REPORTED SPECIES IDENTIFICATION

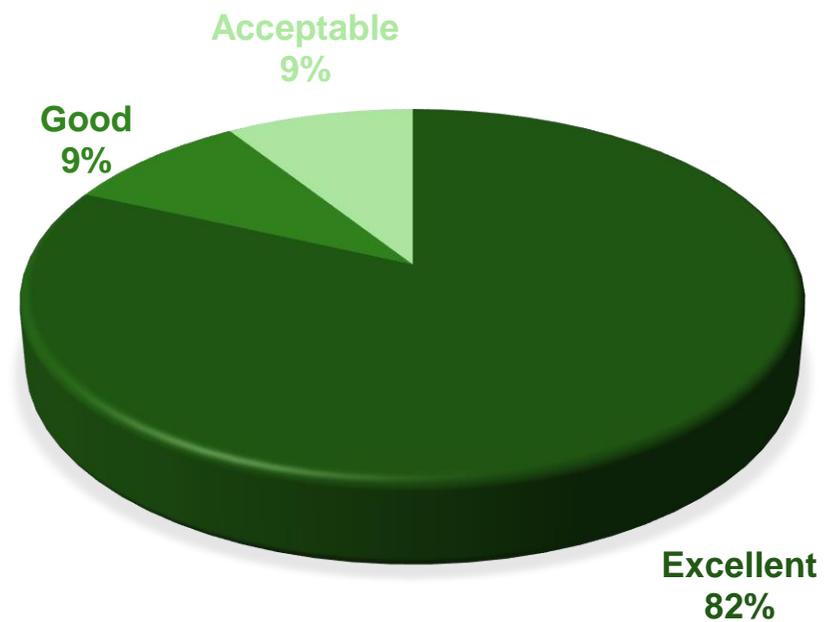
Sample No.	Bacterial species	<i>C. jejuni</i>	<i>C. coli</i>	<i>C. lari</i>	<i>Campylobacter</i> spp. but unable to identify species	Growth of other, not <i>Campylobacter</i>	No growth at all
11 / 27	<i>Campylobacter lari</i>	0+1	0+1	21+19	1+0	0+1	
12 / 24	<i>Campylobacter jejuni</i>	22+21					0+1
13 / 30	<i>Campylobacter coli</i>		22+20	0+1			
14 / 22	Negative					7+18	15+4
15 / 29	Negative	0+1	1+0			8+15	13+6
16 / 28	<i>Campylobacter coli</i>		21+18	0+1		0+2	0+1
17 / 25	<i>Campylobacter lari</i>		0+1	21+18	1+0	0+2	0+1
18 / 23	<i>Campylobacter jejuni</i>	22+17				0+3	0+2
19 / 21	<i>Campylobacter jejuni</i>	21+17				1+3	0+2
20 / 26	<i>Campylobacter jejuni</i>	21+18				1+2	0+2

PT 24: PERFORMANCE – SENSITIVITY SPECIES IDENTIFICATION

MINCED CHICKEN MEAT



BOOT SOCK SAMPLES



PT 24: EDUCATIONAL SAMPLES

Sample No.	Bacterial species	<i>C. jejuni</i>	Both <i>C. jejuni</i> and <i>C. lari</i>	<i>C. lari</i>	<i>C. upsaliensis</i>	<i>C. helveticus</i>	<i>C. lanienae</i>	<i>C. cuniculorum</i>	<i>Campylobacter</i> spp. but unable to identify species	No <i>Campylobacter</i> detected
31	<i>Campylobacter lanienae</i>	1		1			11		7	6
32	<i>Campylobacter helveticus</i>					9		1		17
33	<i>Campylobacter upsaliensis</i>				17				1	9
34	<i>Campylobacter lari</i> <i>Campylobacter jejuni</i>	11	11	4					1	

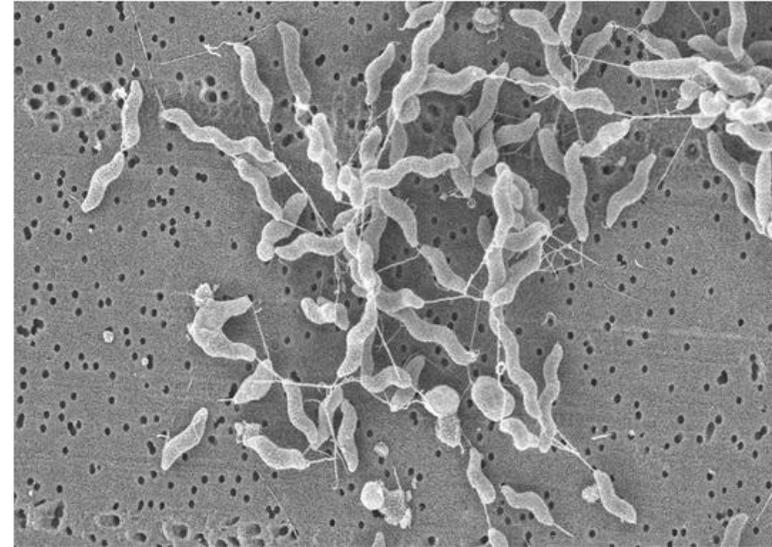
PT 24: OVERALL SENSITIVITY AND PERFORMANCE RATE FOR EDUCATIONAL SAMPLES

Sample No.	Campylobacter species	Sensitivity in detection	Sensitivity in species id	Combined performance rate
31	<i>C. lanienae</i>	77.8%	52.4%	59.3%
32	<i>C. helveticus</i>	37.0%	90.0%	35.2%
33	<i>C. upsaliensis</i>	66.7%	94.4%	64.8%
34	<i>C. lari</i> + <i>C. jejuni</i>	100.0%	68.5%	84.3%
All		70.4%	73.0%	60.9%

COMMENTS AND QUESTIONS

- Sock samples:
 - How much liquid should be used to one sock sample?
 - Which procedure (A, B, C) is most adequate?





THANK YOU!

