Results and analysis of performance in proficiency test 31 and 32

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EURL-*Campylobacter* Workshop 2022

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

Number of participants

Year	2022	2021	2020	2019	2018	2017	2016	2015	2014	2013
	PT 31	PT 29	PT 26	PT 23	PT 21	PT 19	PT 17	PT 15	PT 13	PT 11
Enumeration	34	33	33	35	37	36	36	36	35	36
	PT 32	PT 30	PT 27	PT 24	PT 22	PT 20	PT 18	PT 16	PT 14	PT 12
Detection & species id	31	36	29	33	31	34	33	32	36	34

PT 31 – Enumeration (and species identification)

Proficiency test no. 31

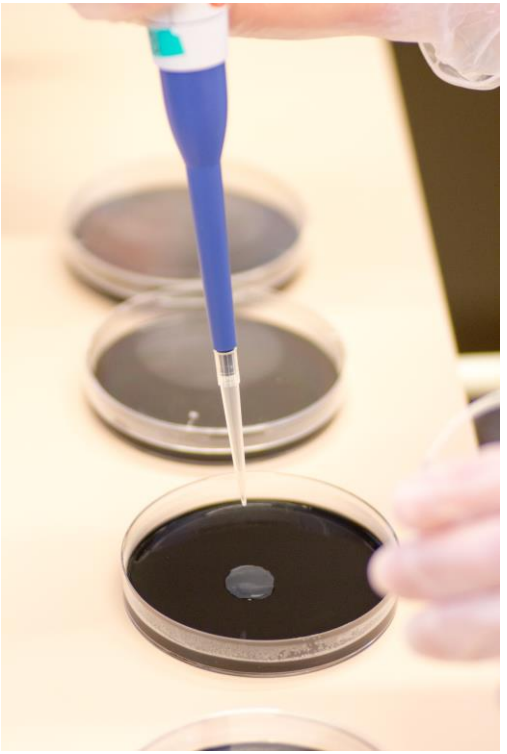
Objective: to assess the performance of the NRLs to enumerate (and voluntary species identify) *Campylobacter* in chicken skin

- Enumeration and confirmation of *Campylobacter* spp. in chicken skin
- Species identification of *Campylobacter* (voluntary)
- Recommended method ISO 10272-2:2017, but other methods allowed
- Should allow enumeration of between 10 and 10^5 cfu *Campylobacter*/g chicken skin

PT 31: Contents and procedure

- One bag of approx. 120 g chicken skin to be divided into 10 portions of 10 g
- 10 vials with freeze-dried sample (with or without *Campylobacter*)
- Make an initial dilution of 10-1 and homogenise
- Follow the method(s) of choice for
  - enumeration
  - species identification (voluntary)

  } of *Campylobacter* spp.



PT 31: Description of the 10 vials

Sample No.	Species	Level (log cfu/vial)		Batch No.
1	*C. coli*	4.71		SLV367
2	*C. coli*	3.76		SLV334
3	*C. coli*	4.71		SLV367
4	*C. jejuni*	4.64		SLV336
5	*C. jejuni* / *Escherichia coli*	4.11	3.56	SLV313
6	*C. lari*	2.81		SLV297
7	*C. lari*	5.15		SLV335
8	*Escherichia coli*		4.19	SLV369
9	*C. jejuni* / *Escherichia coli*	4.11	3.56	SLV313
10	Negative			SLV337

PT 31: Quality control

- Vials produced and tested for homogeneity and stability by the Swedish Food Agency
- Before selection for the PT, the EURL did enumeration of three vials per batch together with chicken skin to ensure levels and functionality
- The EURL performed the complete test the day after dispatch and after the final date to start the analysis
- The EURL did additional enumerations on vials with *Campylobacter* to test stability during transport conditions

Test of stability during transport conditions

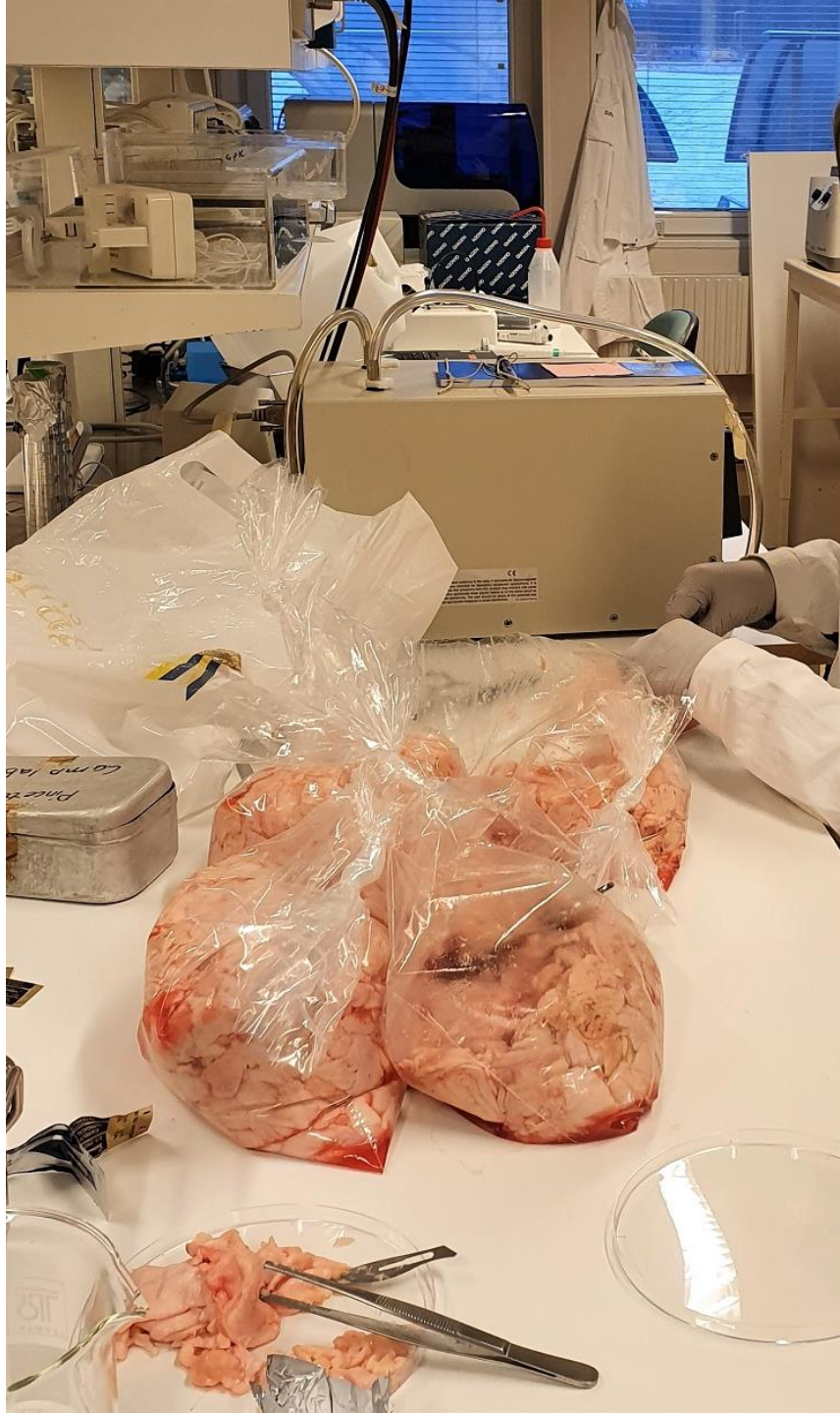
Test occasion	Storage condition	No. of samples tested
Before dispatch	Best case	Each vial with *Campylobacter* x 2
Before dispatch	Worst case	Each vial with *Campylobacter* x 3
Day after dispatch	Best case	The complete test
One week after dispatch	Worst case	Each vial with *Campylobacter* x 2
Two weeks after dispatch	Best case	The complete test
Two weeks after dispatch	Worst case	Each vial with *Campylobacter* x 2

Best case: 5 °C for 24 h

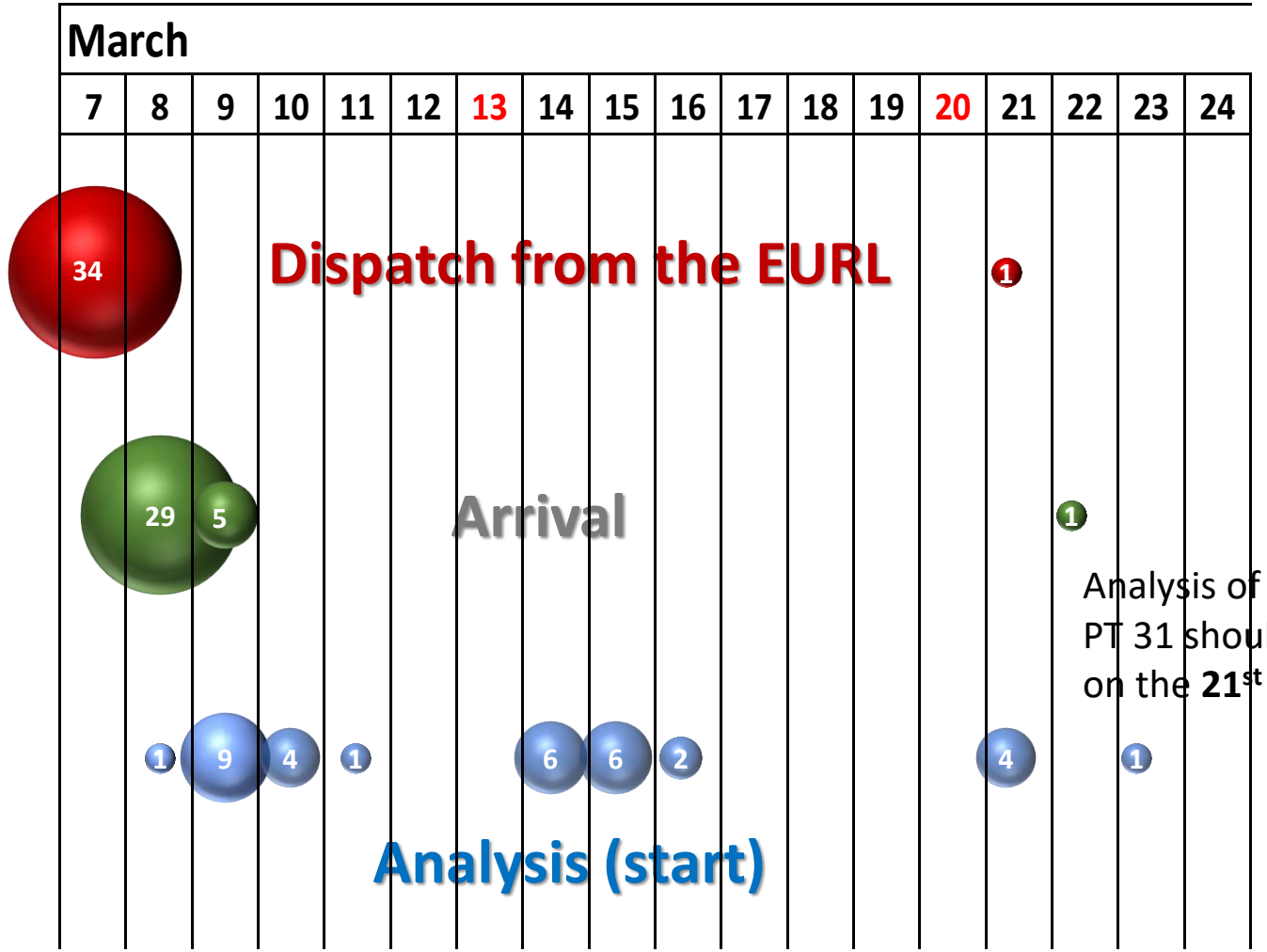
Worst case: 5 °C for 24 h, 15 °C for 24 h and 5 °C for 24 h

PT 31: Preparation of the chicken skin

- Chicken thigh skin delivered from a slaughterhouse with very low level of *Campylobacter*-positive flocks
- Tested in triplicates with enrichment in Bolton and Preston broth, as well as direct streak on mCCDA and all samples tested negative for presence of *Campylobacter*
- Tested negative for presence of background flora on mCCDA
- Cut into pieces and divided into portions of about 120 g
- Stored at −20 °C until distribution



PT 31: Time to arrival & start of analysis



Analysis of the samples included in PT 31 should be started at the latest on the **21st of March 2022**.

PT 31: How was performance calculated?

- The Median Absolute Deviation (MADe) to calculate performance
- σMADe = MADe × 1.4826
- *Campylobacter*-containing samples
  - Results within participants' median ±2σMADe = 2 points
  - Results between ±2σMADe and ±3σMADe = 1 point
  - Results outside ±3σMADe = 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

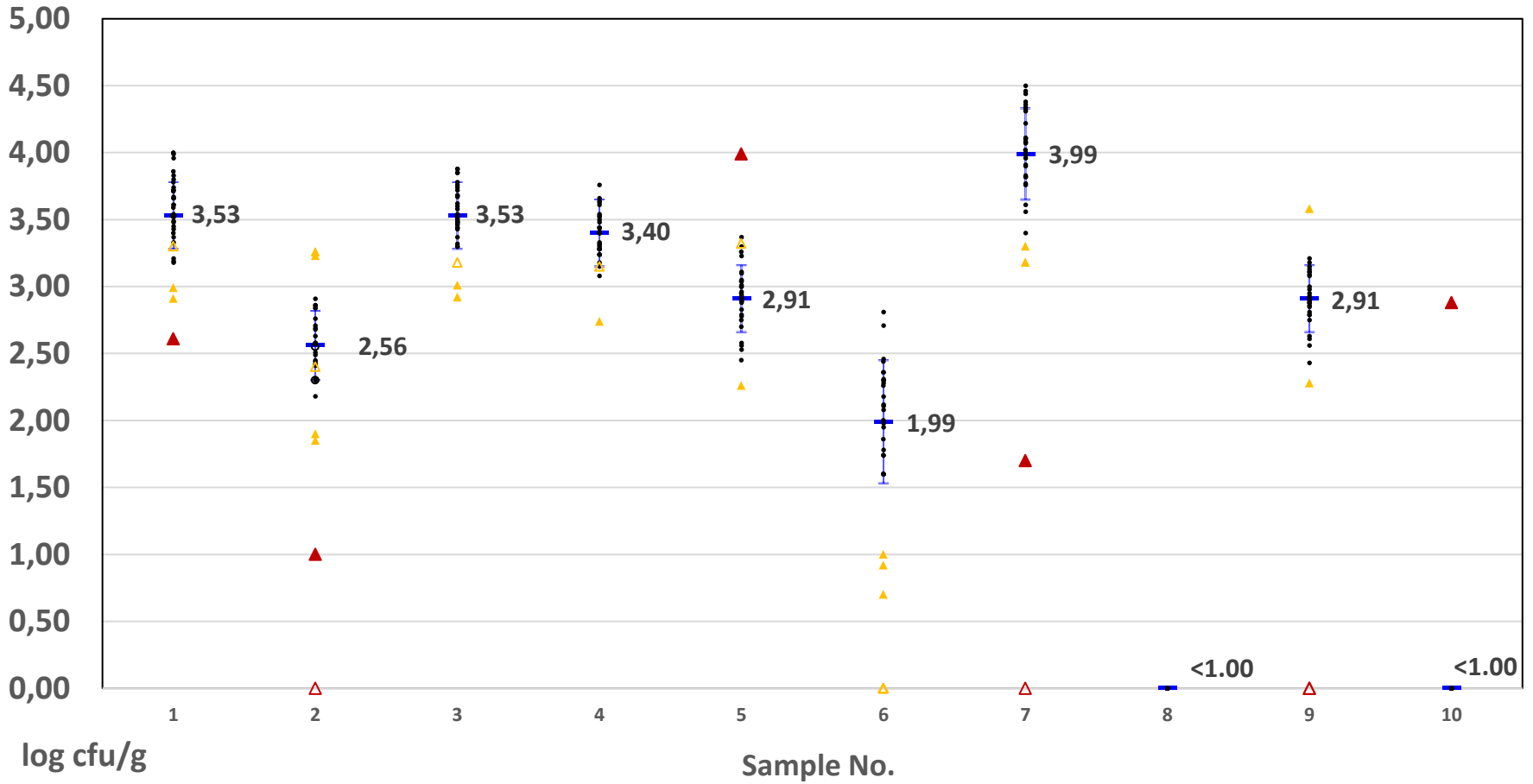
Grade	Scoring limits	
Excellent	20	95.1–100%
Good	17–19	85.0–95.0%
Acceptable	14–16	70.0–84.9%
Needs improvement	12–13	57.0–69.9%
Poor	<12	<57.0%

PT 31: How was performance calculated?

Adaptions because of use of duplicates and low levels

- Duplicate vials (1 and 3, 5 and 9)
  - Median and σMADe calculated for 1) each single sample, 2) each pair of samples
  - For performance evaluation: duplicate values used,
    thus the same scoring limits applicated for both samples in a pair
- For sample 6, the -3σMADe limit below 1.0 log cfu/g
  - minimum score given for results below this level adjusted
  - included results where no campylobacters were reported

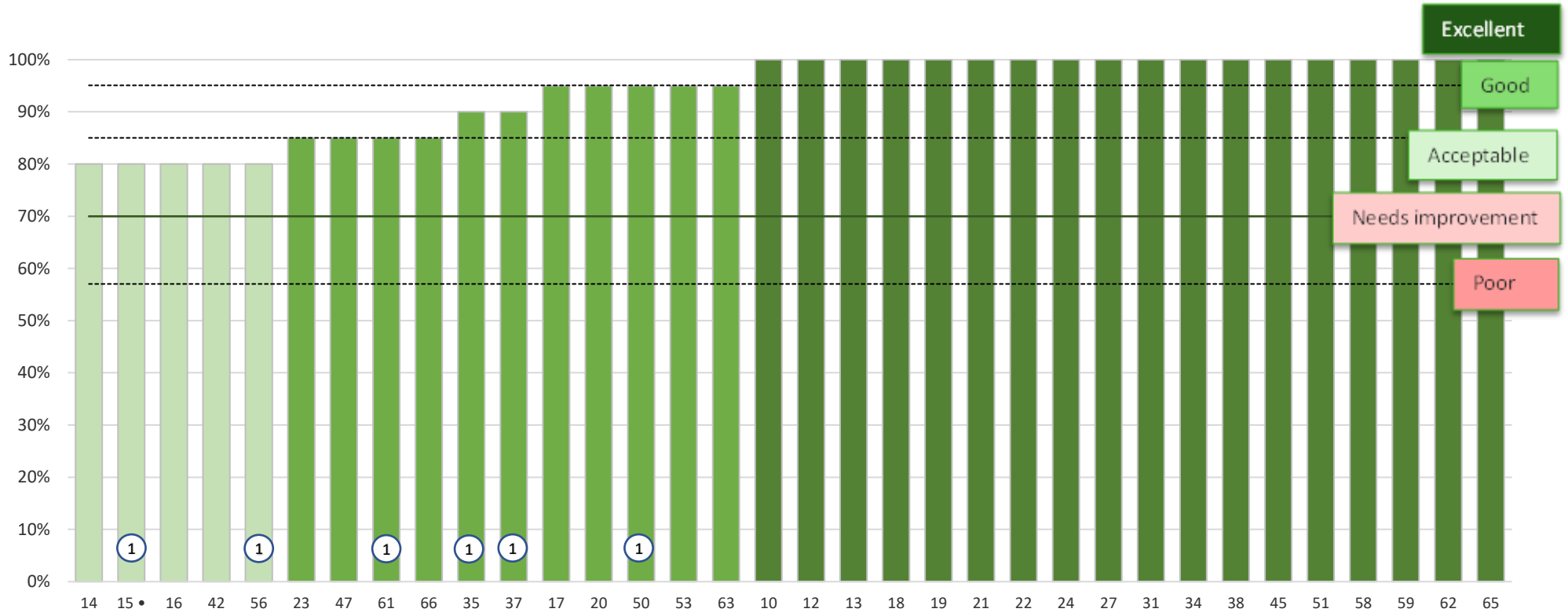
PT 31: Results of enumeration



Variability in PT enumeration results

		max-min diff (between labs)				MADe in PT			
Year	*PT*	*max*	*min*	*mean*	*median*	*max*	*min*	*mean*	*median*
2017	**19**	5.90	2.19	**3.54**	3.23	0.37	0.23	**0.30**	0.29
2018	**21**	4.06	1.80	**3.02**	3.31	0.49	0.17	**0.30**	0.28
2019	**23**	2.48	1.27	**1.88**	1.94	0.24	0.19	**0.21**	0.22
2020	**26**	3.36	0.92	**1.89**	1.75	0.32	0.13	**0.24**	0.24
2021	**29**	2.65	1.89	**2.17**	2.08	0.45	0.29	**0.37**	0.38
2022	**31**	3.50	0.96	**1.94**	1.92	0.31	0.12	**0.18**	0.16
	mean	3.86	1.43	2.45	2.43	0.35	0.17	0.25	0.24

Performance PT 31



PT 31: Species identification (voluntary)

Content of sample (vial)	*C. jejuni*	*C. coli*	*C. lari*	No growth	Growth of other
1. ***C. coli***		30			
2. ***C. coli***		29			1
3. ***C. coli***	2	28			
4. ***C. jejuni***	30				
5. ***C. jejuni* + *E. coli***	28	1	1		
6. ***C. lari***			28	2	
7. ***C. lari***	1		29		
8. ***E. coli***				3	27
9. ***C. jejuni* + *E. coli***					
10. ***Blank***	1			29	

Measurement uncertainty

- MU values for the samples in PT 31 specified by 20 NRLs
- 16 used the current standard EN ISO 19036:2019, 4 the previous version ISO/TS 19036:2006/Amd 1:2009
- Most values well within the suggested limits for acceptable MU values in the guidance document
- Additional data will be collected in future PTs
- The questionnaire may need some clarifications

PT 32 – DETECTON AND SPECIES IDENTIFICATION

Proficiency test no. 32

The objective was to assess the performance of the NRLs to detect and identify *Campylobacter* species in pig faeces.

- Detection of *Campylobacter* spp. in pig faeces (animal samples)
- Species identification of *Campylobacter*
- 10 samples: 6 low level, 2 high level, 2 negative
- Recommended method was procedure C (direct streak) in ISO 10272-1:2017, but other methods allowed
- Enough faeces provided for performing enrichment (if of interest for the laboratory)

PT 32: Contents and procedure: pig faeces core samples

- A plastic bag with about 120 g of sterilized pig faeces
- 10 freeze-dried vials (with or without *Campylobacter* and/or other bacteria)
- Reconstitute each vial within 5 minutes and prepare the samples within 30 minutes
- Mix each vial with 6 g pig faeces in stomacher bags
- Follow the method(s) of choice for
  - detection
  - species identification

  } of *Campylobacter* spp.

PT 32: Contents and procedure: pig faeces educational samples

- 2 plastic tubes with about 10 g of fresh (naturally contaminated) pig faeces
- Follow the method(s) of choice for
  - detection
  - species identification

  } of *Campylobacter* spp.
- Laboratories free to make adjustments to methods as to increase their chance to find more *Campylobacter* spp.

Description of the 10 vials in PT 32

Sample No.	Species	Level (log cfu/vial)		Level (log cfu/test portion)	Batch No.
11	*C. coli*	4.82 (low)		1.82	SVA068
12	Negative				
13	*E. coli*		4.80		SVA061
14	*C. jejuni*	5.27 (low)		2.27	SVA065
15	*C. coli*	4.82 (low)		1.82	SVA068
16	*C. coli*	4.82 (low)		1.82	SVA068
17	*C. coli*	7.10 (high)		4.10	SVA072
18	*C. jejuni*	5.27 (low)		2.27	SVA065
19	*C. lari* / *E. coli*	6.66 (high)	5.85	3.66	SVA070
20	*C. jejuni*	5.27 (low)		2.27	SVA065

PT 32: Quality control

- Vials produced and tested for homogeneity and stability by the EURL
- Before selection for the PT, the vials were tested together with pig faeces to ensure functionality
- The complete test was performed the day after dispatch and after the final date to start the analysis
- Additional tests were done on vials with *Campylobacter* to test stability during transport conditions

Test of stability during transport conditions

Test occasion	Storage conditions	Procedure A= Bolton B= Preston C= Direct streak	No. of samples tested	Result
Before dispatch	Best case	A + B + C	Vials with Campylobacter × 3	+
Before dispatch	Worst case	A + B + C	Vials with Campylobacter × 3	+
Just after dispatch	Best case	C	The complete test	+
1 week after dispatch	Best case	C	The complete test	+
1 week after dispatch	Worst case	B +C	The complete test	+

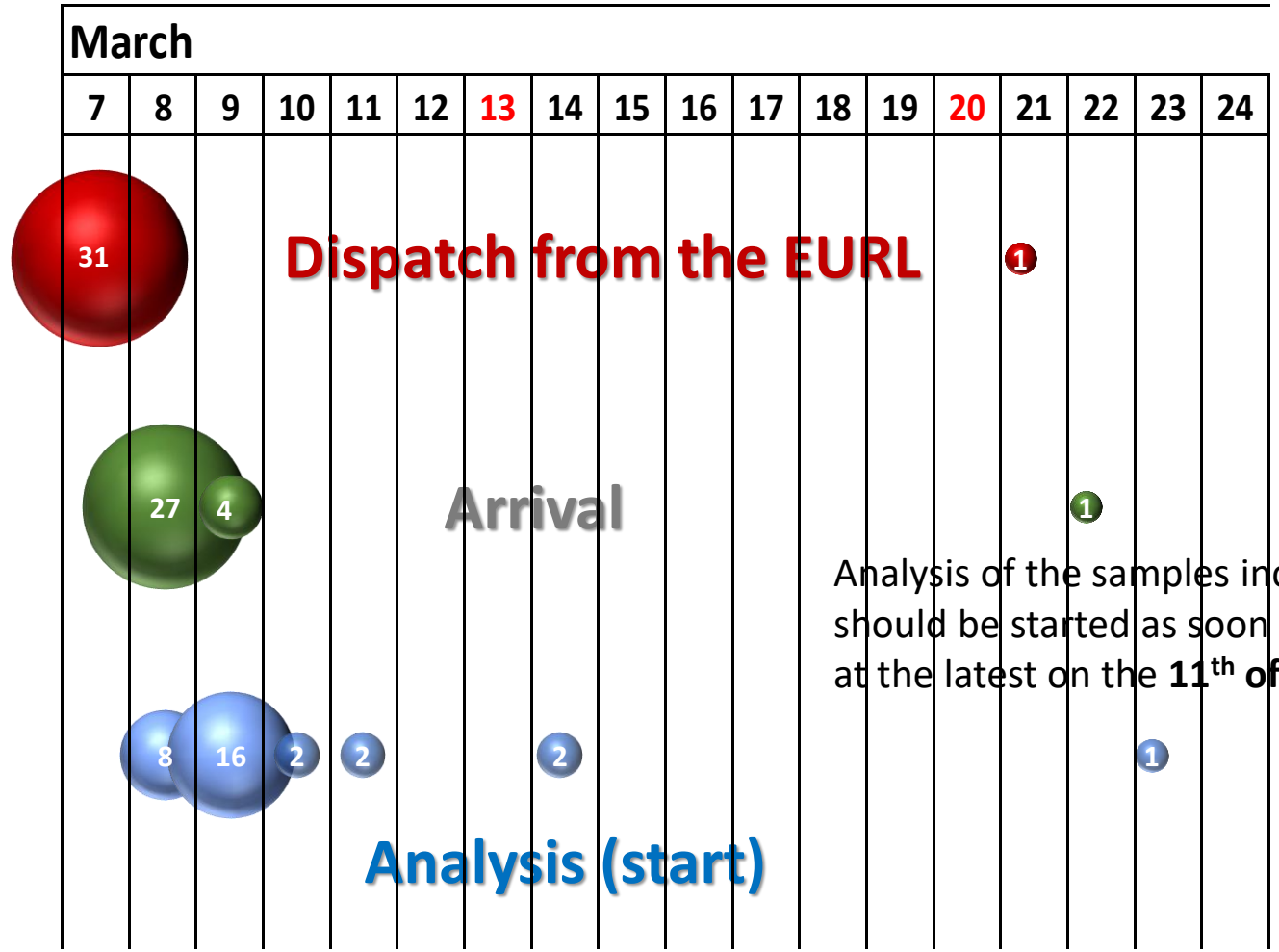
Best case: 5 °C for 24 h

Worst case: 5 °C for 24 h, 15 °C for 24 h and 5 °C for 24 h

PT 32: Preparation of the faeces

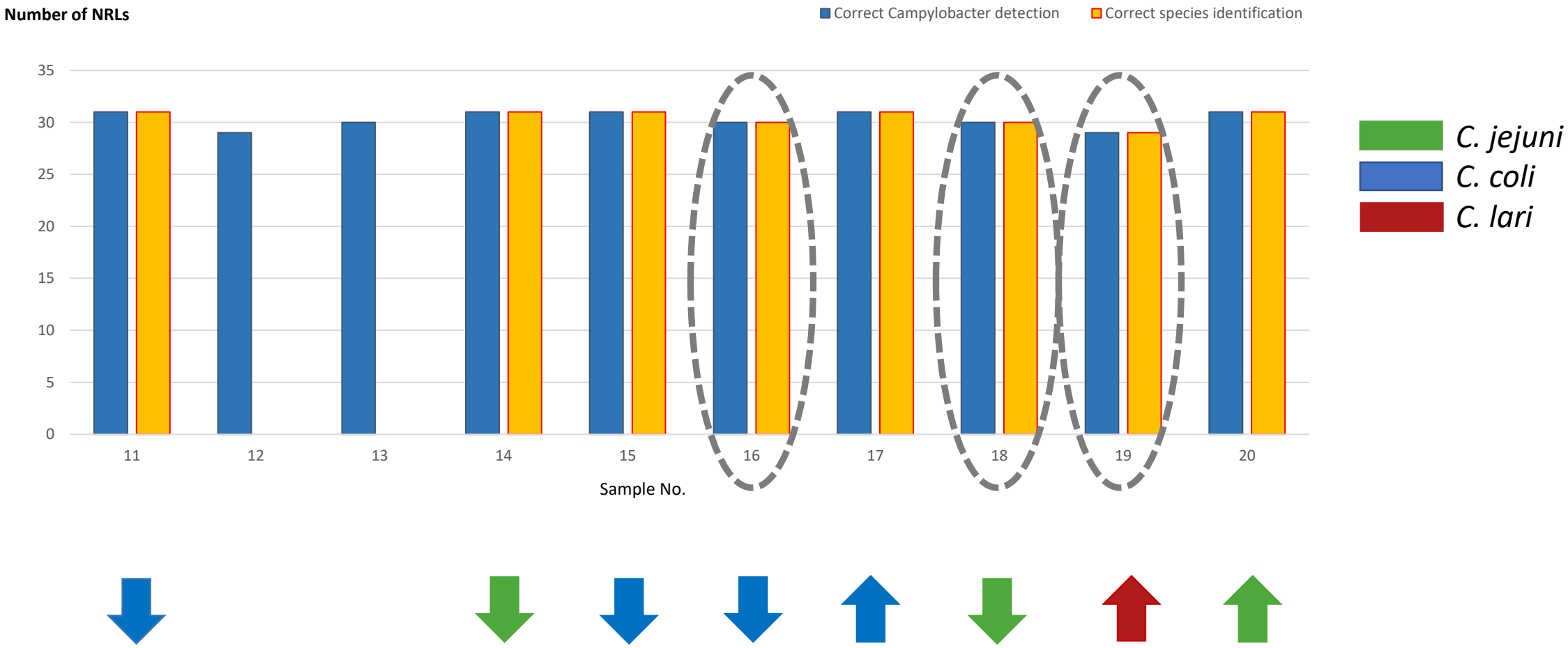
- Pig faeces for **core samples** were collected from a local pig farm three months before the PT
- Autoclaved and tested negative for presence of *Campylobacter* spp. Stored at −20 °C until distribution
- Pig faeces for **educational samples** were collected at two different compartments at a local pig farm three days before dispatch. Stored +4 °C until distribution.

PT 32: Time to arrival & start of analysis

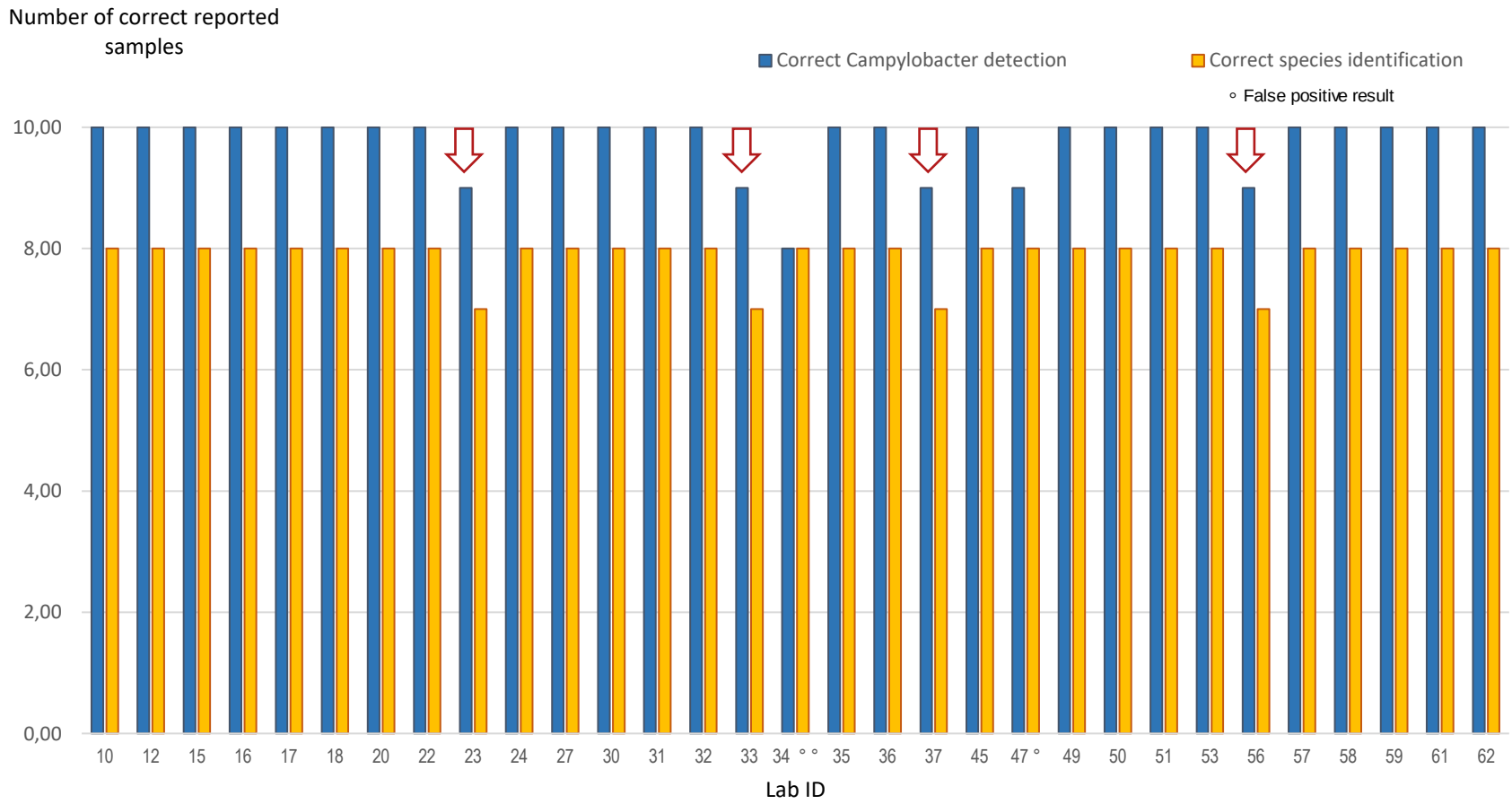


Analysis of the samples included in PT 32 should be started as soon as possible and at the latest on the **11th of March 2022**.

PT 32: correct reported results per sample



PT 32: Correct reported results per lab

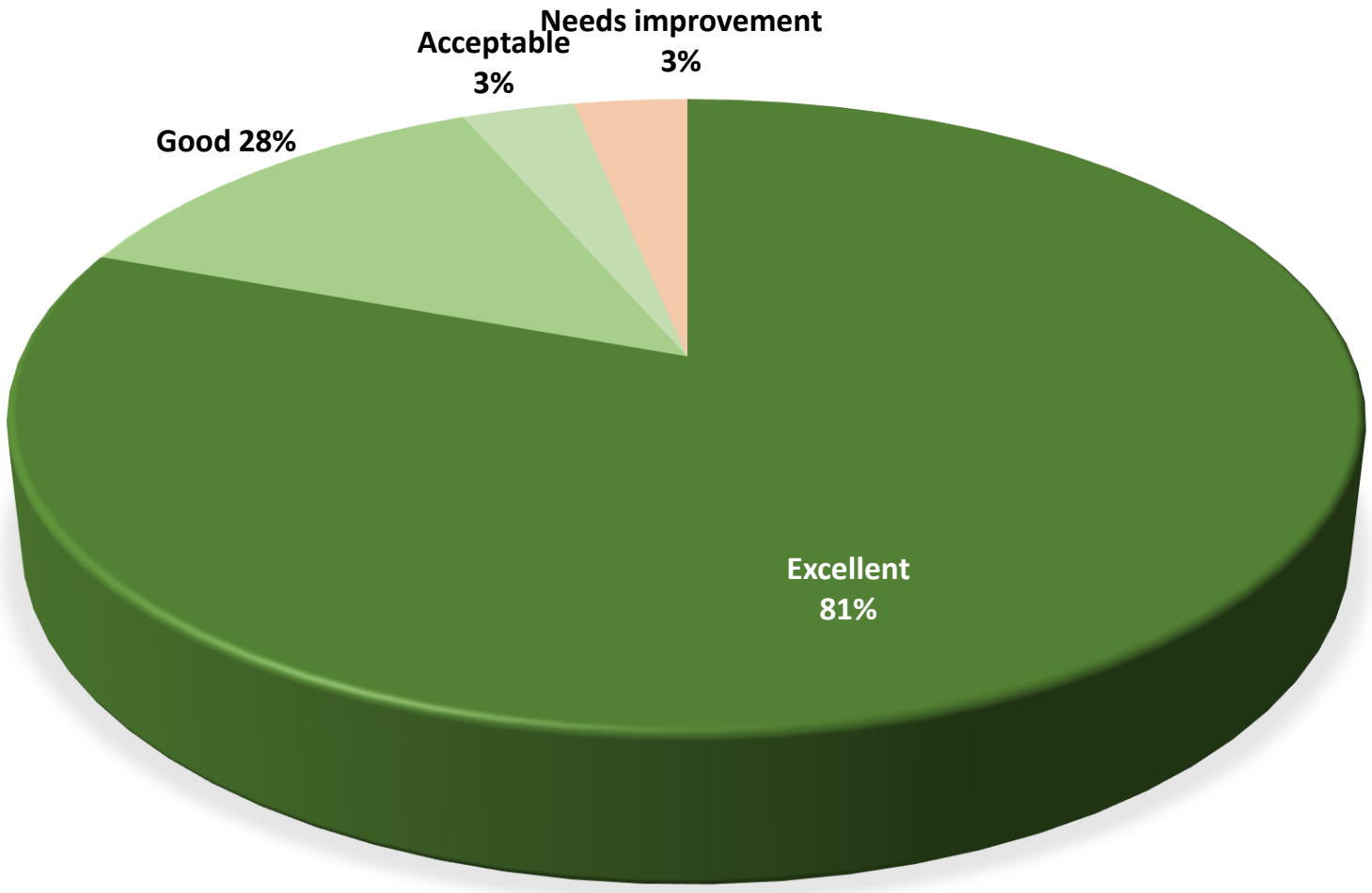


PT 32: Combined performance grade

Maximum number of false positive results, and the lower limits of sensitivity (Se) and accuracy (Acc), applied for each combined performance grade

	Category of samples	Measures on the lower limit of each grade		
Performance grade	**Number of false positives**	**Se total**	**Acc**	**Spec id**
Excellent	0	95%	95.1%	95%
Good	0	85%	90%	85%
Acceptable	1	70%	80%	70%
Needs improvement	2	57%	70%	57%

PT 32: Overall performance in detection of *Campylobacter*

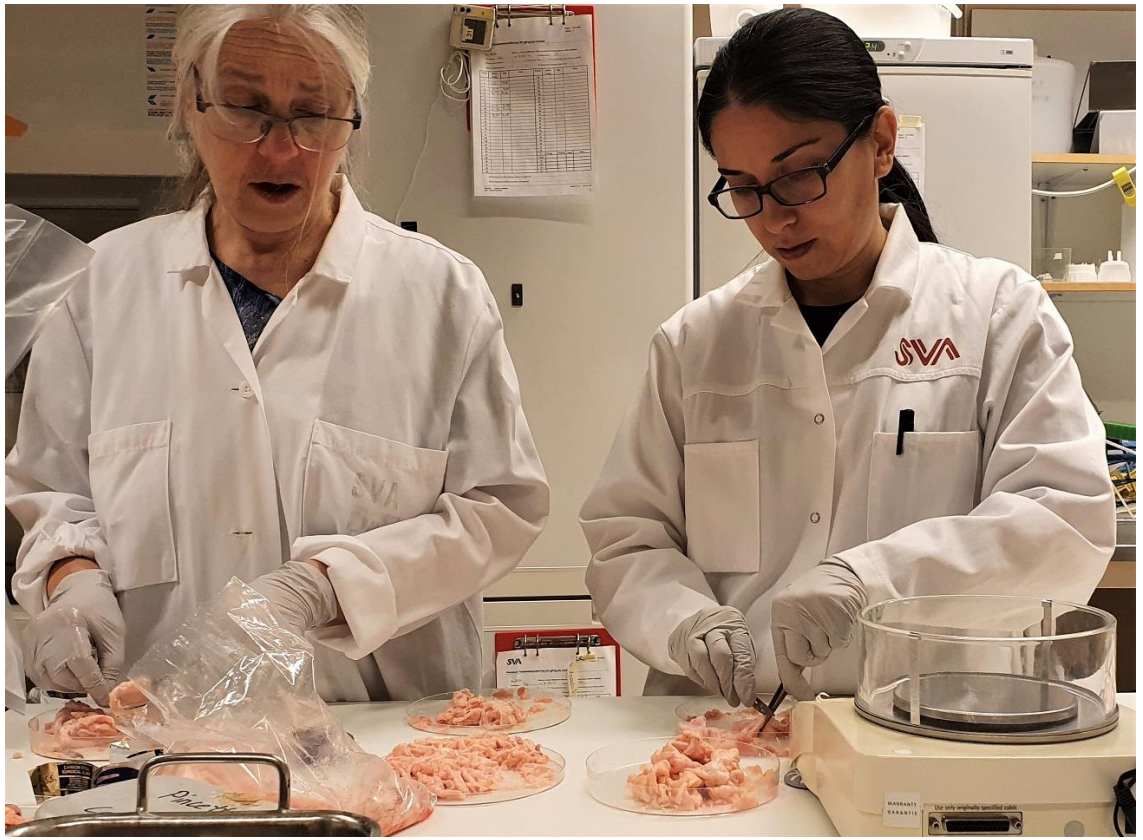


PT 32: Species identification

- No misidentifications
- 100% excellent performance

PT 32: Results educational samples

- Results reported from 27 NRLs
- Sample No. 21
  - **23 detected *Campylobacter* spp.**, 4 reported growth of other bacteria only
  - 17 *C. jejuni*, 14 *C. hyointestinalis*, 1 *C. lanienae*, 1 *C. coli*
  - 3 reported *Campylobacter* but unable to specify
  - 8 NRLs reported 2 different *Campylobacter* species, and 1 NRL 3 species
- Sample No. 22
  - **16 detected *Campylobacter* spp.**, 11 reported growth of other bacteria only
  - 7 *C. hyointestinalis*, 6 *C. jejuni*, 4 *C. lanienae*, 1 *C. mucosalis*
  - 3 reported *Campylobacter* but unable to specify 3
  - 5 NRLs reported 2 different *Campylobacter* species



Thank you for listening!