Results and analysis of performance proficiency tests 39 and 40

EURL-*Campylobacter* workshop 2025

Helena Höök, EURL-*Campylobacter*, 30 September

SWEDISH VETERINARY AGENCY

EURL Campylobacter

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

Number of PT participants

Year	2025	2024	2023	2022	2021	2020	2019	2018	2017	2016
	PT 39	PT 36	PT 34	PT 31	PT 29	PT 26	PT 23	PT 21	PT 19	PT 17
Enumeration	35	35	35	34	33	33	35	37	36	36
	PT 40	~~PT 37~~	PT 35	PT 32	PT 30	PT 27	PT 24	PT 22	PT 20	PT 18
Detection & species id	34	-	32	31	36	29	33	31	34	33

Proficiency test No. 39 Enumeration (and species identification)

- Outline
- Methods
- Results and performance of enumeration
- Results and performance of species identification

PT 39: Outline

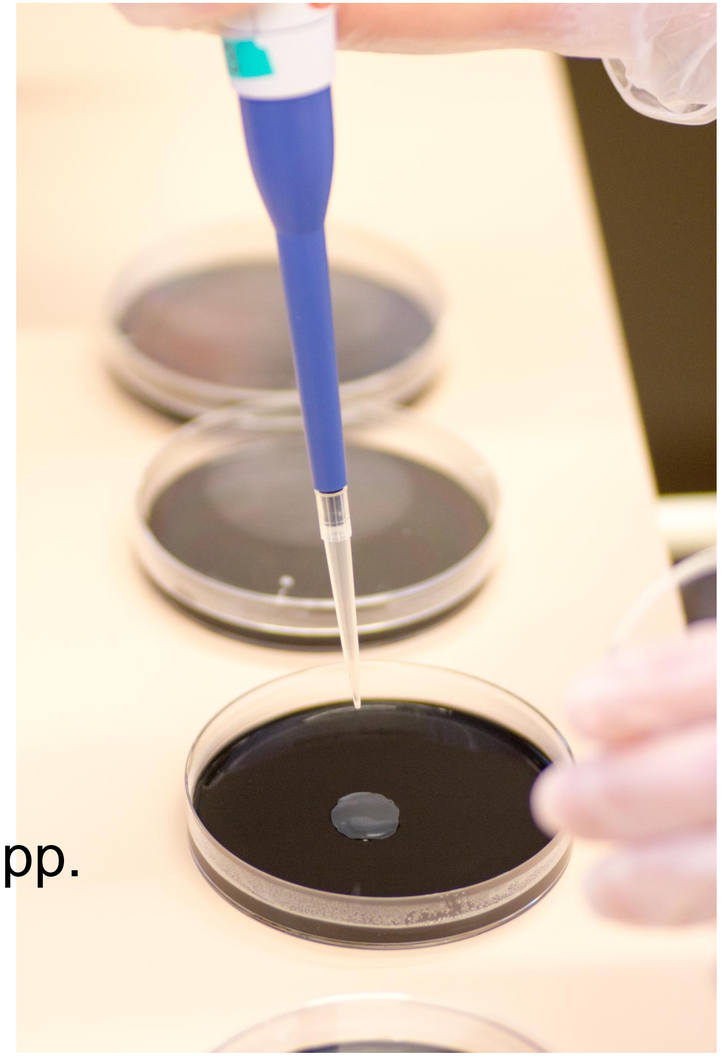
Objective: to assess the performance of the NRLs to enumerate (and voluntary species identify) *Campylobacter* in chicken skin

- **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 39: Contents and procedure

- One plastic bag of about 130 g frozen 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 39: Description of the 10 vials

Sample No.	Species	Level (log cfu/vial)		Batch No.
1	*Campylobacter coli*	4.77		SLV367
2	*Campylobacter jejuni*	3.24		SLV403
3	*Escherichia coli*		4.80	SVA061
4	*Campylobacter coli*	4.77		SLV367
5	*Campylobacter coli*	4.77		SLV367
6	Negative			
7	*Campylobacter jejuni*	3.95		SLV401
8	*Campylobacter jejuni*	3.95		SLV401
9	*Campylobacter coli*	5.04		SLV375
10	*Campylobacter jejuni*	3.24		SLV403

PT 39: Quality control

- Vials produced and tested for homogeneity and stability by the Swedish Food Agency or the EURL
- 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 enumerations on vials with *Campylobacter* in chicken skin to test stability during "best case" and "worst case" transport conditions
- Max-min diff 0.79 $\log_{10}$ cfu or lower

Test of stability during transport conditions

Test occasion	Storage condition	No. of samples tested
Before dispatch	Best case	Each vial batch with *Campylobacter* × 2
Before dispatch	Worst case	Each vial batch with *Campylobacter* × 2
Two weeks after dispatch	Worst case	Each vial batch with *Campylobacter* × 2

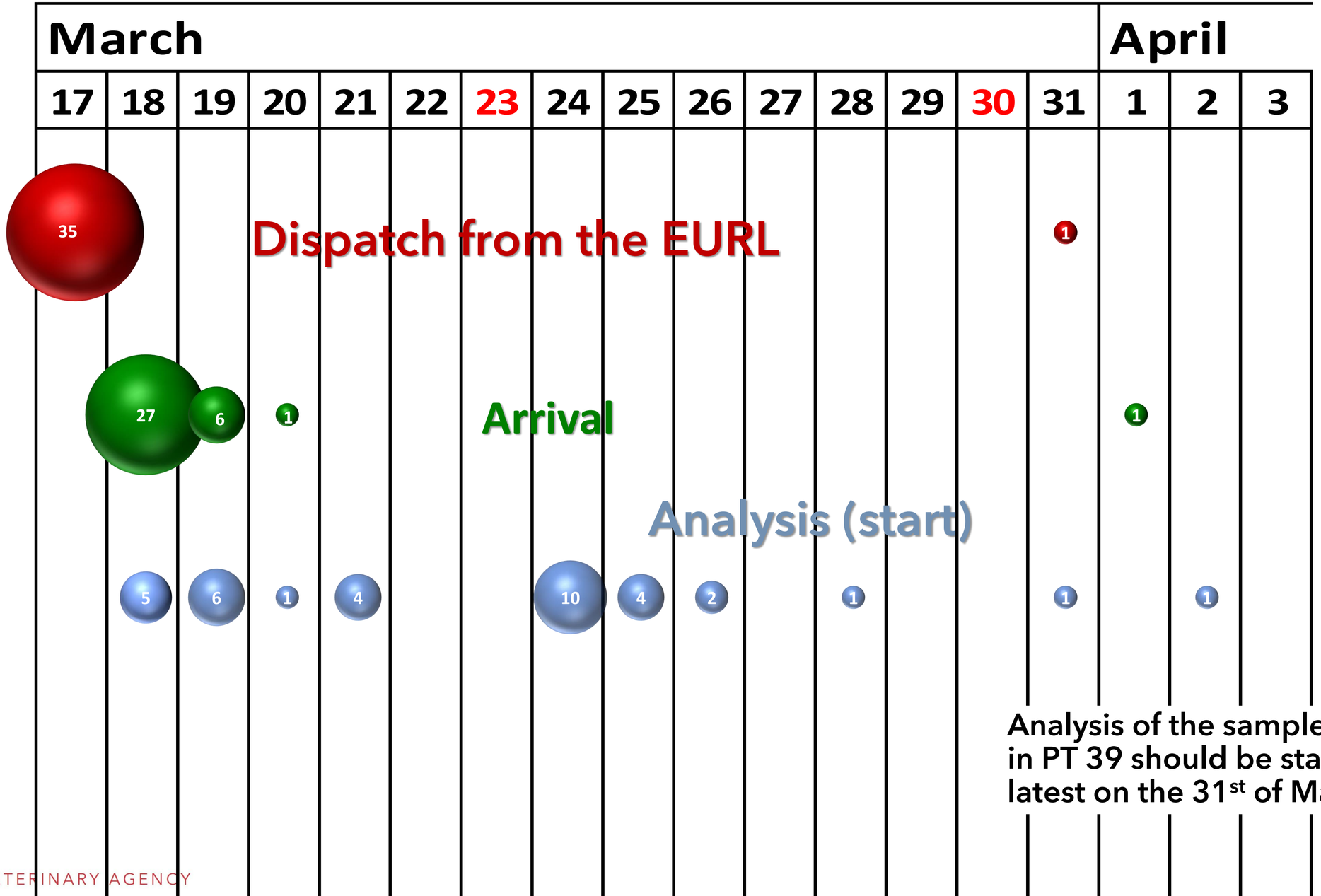
Best case: Box with freezing blocks, room temperature 24 h

Worst case: Box with freezing blocks, room temperature 48 h

PT 39: Preparation of the chicken skin

- Chicken thigh skin delivered from a slaughterhouse with low level of *Campylobacter*-positive flocks and a farm with no positive flocks for more than 5 months
- On arrival, tested in triplicate with streaking on mCCD and Butzler agar after enrichment in Bolton and Preston broth
- All samples tested negative for presence of *Campylobacter* but moderately with background flora was present
- Cut into pieces and divided into portions of about 130 g
- Stored at −20 °C until distribution

PT 39: Time to arrival & start of analysis



Analysis of the samples included in PT 39 should be started at the latest on the 31st of March 2025.

PT 39: How was performance evaluated?

- The Median Absolute Deviation (MADe) to calculate performance

  $\sigma MADe = MADe \times 1.4826$

- *Campylobacter*-containing samples
  - Results within participants' median ± 2 σMADe = 2 points
  - Results between ± 2 σMADe and ± 3 σMADe = 1 point
  - Results outside ± 3 σMADe = 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 39: How was performance calculated?

Adaptations because of homogenous results and use of duplicates/triplicates

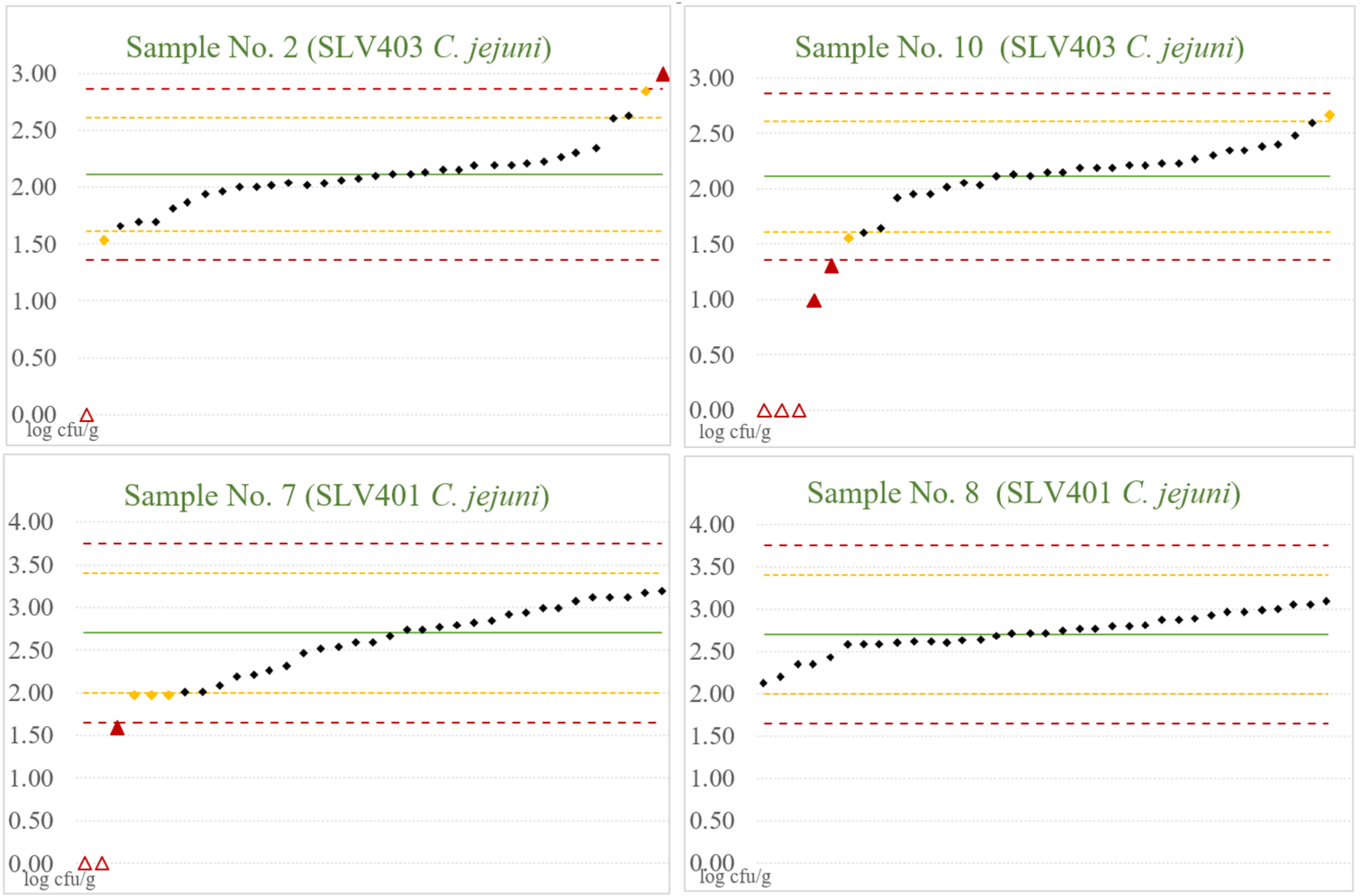
- Homogeneous results (2 and 10)
  - σMADe adjusted to 0.25 $\log_{10}$ cfu/g, according to the 0.5 $\log_{10}$ rule (ISO 22117:2019)
- Duplicate or triplicate vials (1, 4, and 5, 2 and 10, and 7 and 8)
  - Median and σMADe calculated for 1) each single sample, 2) each group of samples
  - For performance evaluation: grouped values used

    ⇒ the same scoring limits for all samples in a group

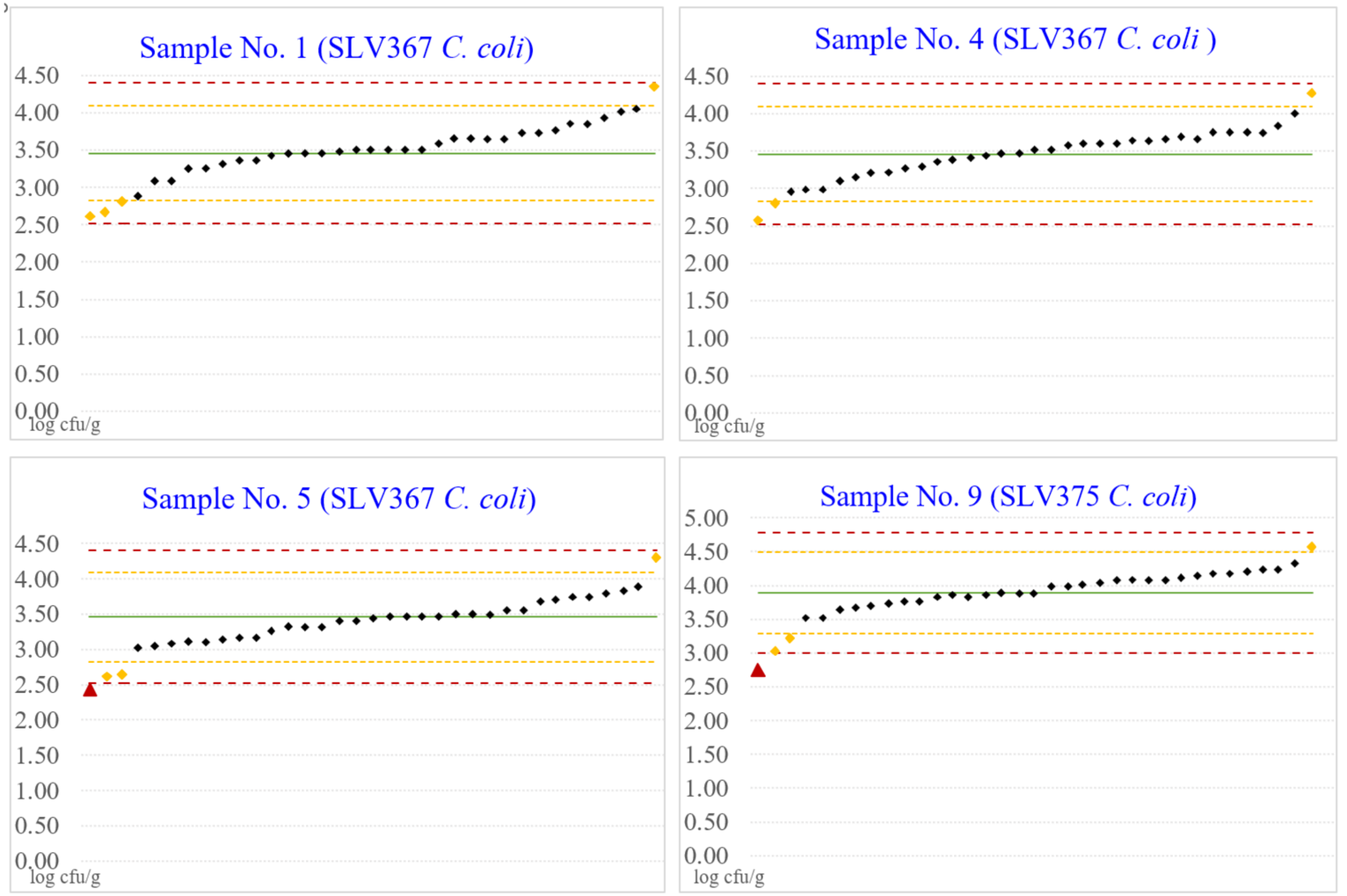
No sample in PT 39 had a −3 σMADe limit below 1.0 $\log_{10}$ cfu/g

- No adjustment of the minimum score for negative results

PT 39: Results of enumeration



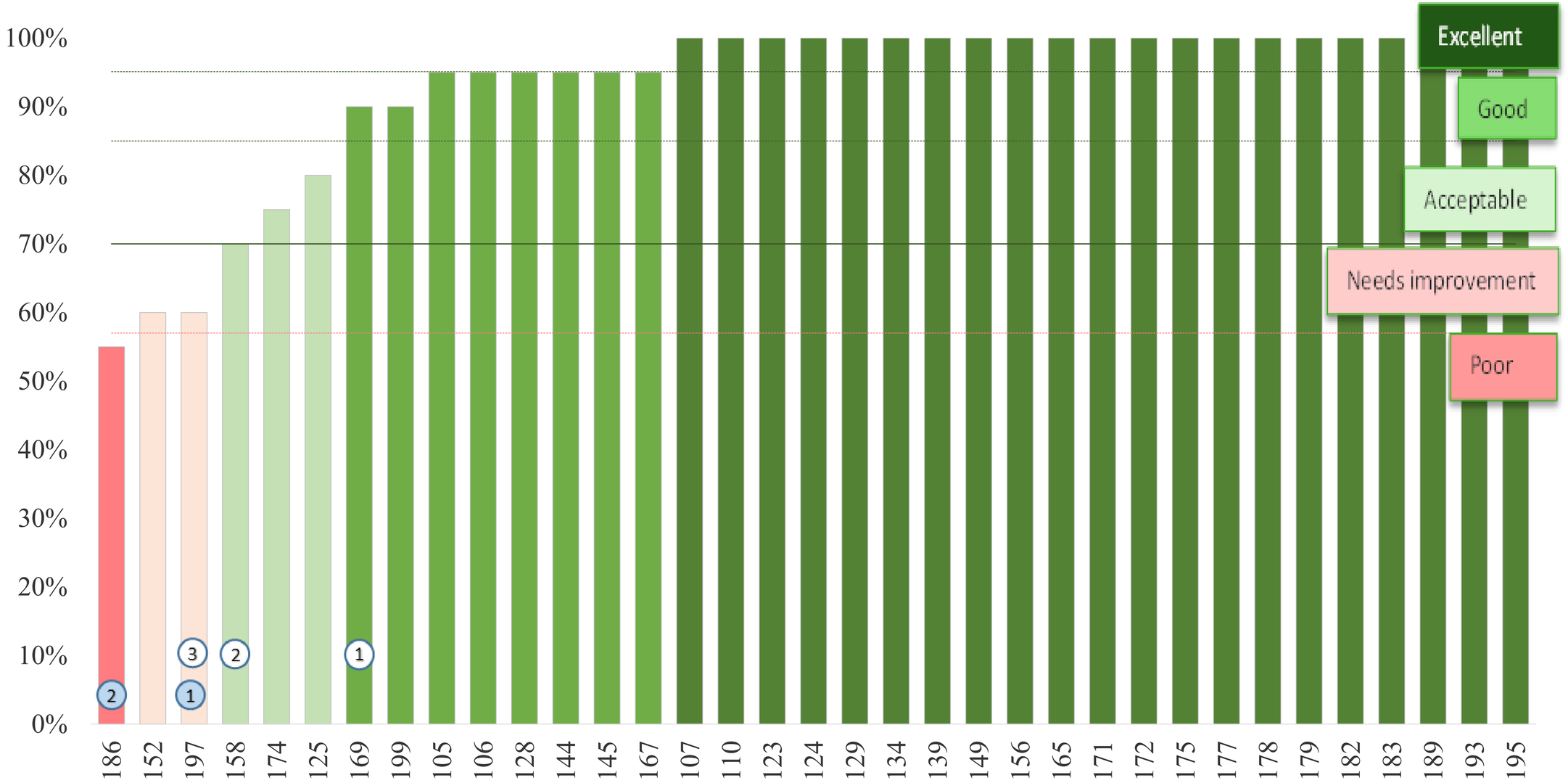
PT 39: Results of enumeration



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**	2.80	0.96	**1.86**	1.92	0.31	0.11	**0.17**	0.15
2023	**34**	3.06	1.42	**2.26**	2.29	0.23	0.14	**0.18**	0.18
2024	**36**	2.00	0.97	**1.62**	1.94	0.26	0.12	**0.17**	0.17
2025	**39**	1.86	0.98	**1.60**	1.69	0.39	0.10	**0.21**	0.21
	mean	3.13	1.37	**2.20**	2.24	0.34	0.16	**0.24**	0.23

PT 39: Performance



PT 39: Species identification (voluntary)

Content of sample (vial)	*C. jejuni*	*C. coli*	*C. lari*	Not able to identify	No growth	Growth of other
1. *Campylobacter coli*		30				
2. *Campylobacter jejuni*	29	1				
3. *Escherichia coli*				1	5	24
4. *Campylobacter coli*		29	1			
5. *Campylobacter coli*		29	1			
6. Negative				1	16	13
7. *Campylobacter jejuni*	29					1
8. *Campylobacter jejuni*	30					
9. *Campylobacter coli*		29	1			
10. *Campylobacter jejuni*	28					2

PT 40 – detecton and species identification

Proficiency test no. 40

The objective was to assess the performance of the NRLs to detect and identify *Campylobacter* species in chicken caecal content.

- Detection of *Campylobacter* spp. in chicken caecal content (animal samples)
- Species identification of *Campylobacter*
- 18 core samples: 6 low level, 6 high level, 6 negative (mandatory)
- 2 educational samples (voluntary)
- Each sample mimicking a sample pooled from up to 30 chicken caeca
- Recommended method was procedure C (direct plating) according to ISO 10272-1:2017, but other methods allowed

PT 40: Contents and procedure: chicken caecal content

- 18/20 numbered plastic tubes with 6 ml of caecal material
- 10 freeze-dried vials (with or without *Campylobacter* and/or other bacteria)
- Mix each vial with the corresponding caecal content tube up to a total volume of 10 ml
- Follow the method(s) of choice for

  detection

  species identification

  } of *Campylobacter* spp.

Description of the 18 core sample vials in PT 40

Sample No.	Content in vial	Batch No.	Level	log cfu/vial	SD (log/cfu)
11	*Escherichia coli*	SVA096		5.81	0.05
12	*Campylobacter coli*	SVA083	high	5.52	0.05
13	*Campylobacter lari*	SVA087	low	4.43	0.06
14	*Campylobacter jejuni*	SVA090	high	6.43	0.05
15	*Escherichia coli*	SVA096		5.81	0.05
16	*Campylobacter jejuni*	SVA085	low	4.00	0.06
17	Negative				
18	*Campylobacter jejuni*	SVA085	low	4.00	0.06
19	*Campylobacter coli*	SVA083	high	5.52	0.05
20	Negative				
21	*Campylobacter jejuni*	SVA090	high	6.43	0.05
22	Negative				
23	*Campylobacter jejuni*	SVA085	low	4.00	0.06
24	*Campylobacter coli*	SVA089	low	3.96	0.05
25	*Escherichia coli*	SVA096		5.81	0.05
26	*Campylobacter coli*	SVA081	high	5.01	0.08
27	*Campylobacter lari*	SVA087	low	4.43	0.06
28	*Campylobacter coli*	SVA081	high	5.01	0.08

PT 40: Quality control

- Vials produced and tested for homogeneity and stability by the EURL
- Each combination of vial batch and matrix tested under various transport conditions
- Procedure C (direct streak) alone or in combination with procedure A and B
- Streaking on mCCD and Butzler agar
- Stability also checked by viable count (VC) on blood agar

Test of stability during transport conditions

Test occasion	Storage condition	Test method	No. of samples tested
Before dispatch	Best case	C	Each vial batch with *Campylobacter* × 2
Before dispatch	Worst case	A+B+C +VC	Each vial batch with *Campylobacter* × 2
One week after dispatch	Worst case	C+VC	Each vial batch with *Campylobacter* × 3

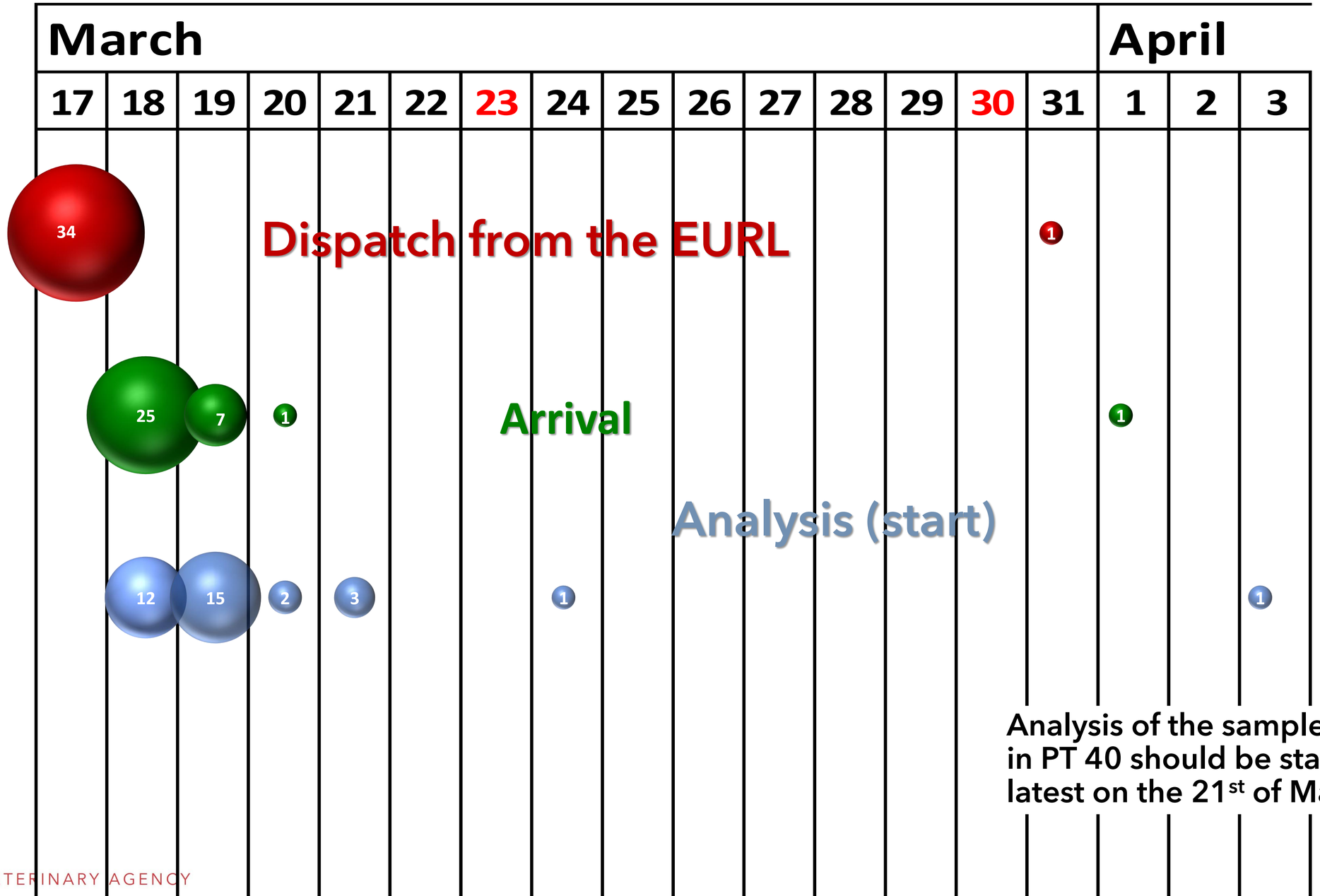
Best case: Box with freezing blocks, room temperature 24 h

Worst case: Box with freezing blocks, room temperature 48 h

PT 40: Preparation of the chicken caecal content

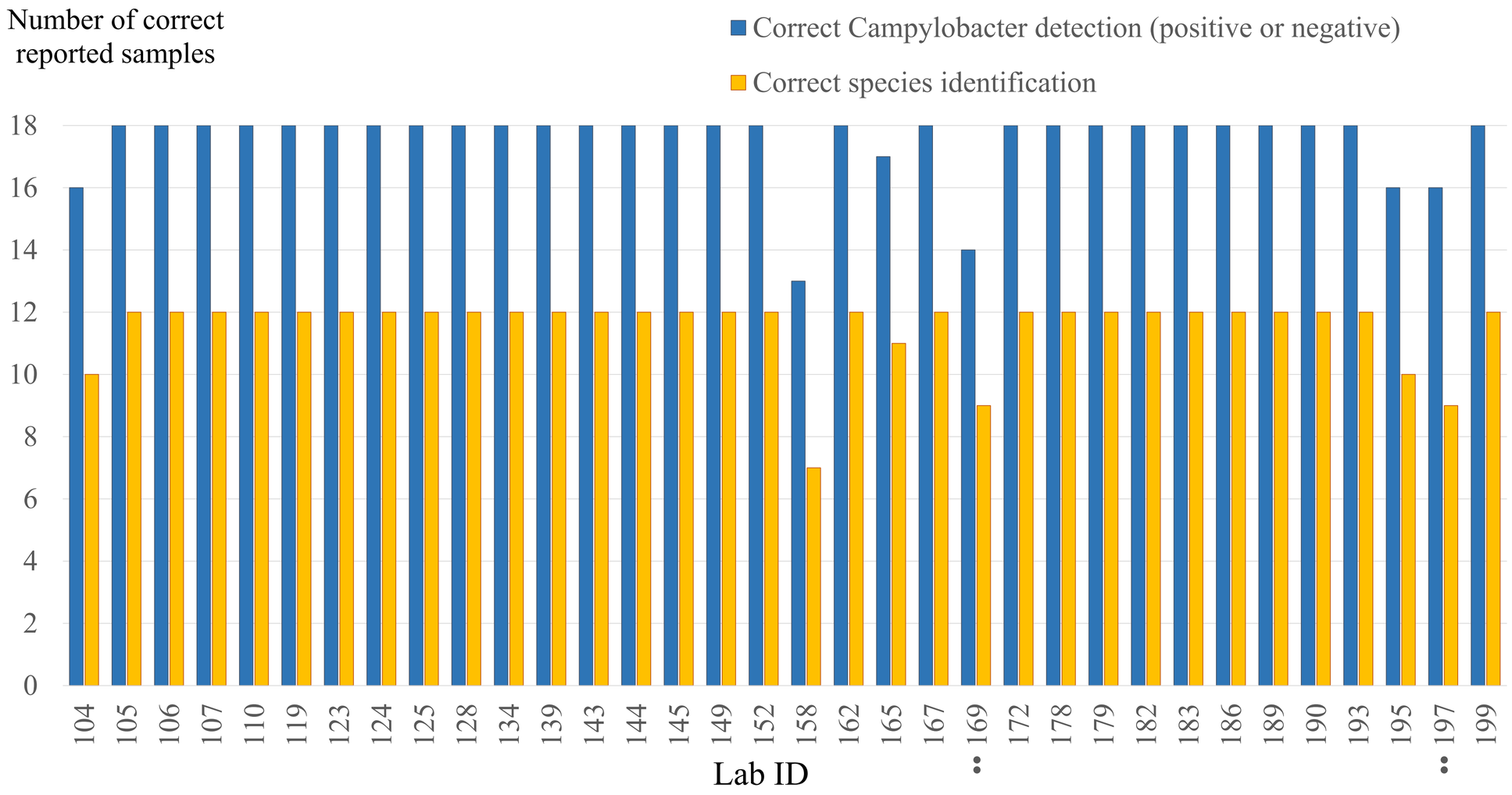
- Chicken caeca delivered from a slaughterhouse with low level of *Campylobacter*-positive flocks and a farm with no positive flocks for more than one year
- On arrival, tested negative for detection of *Campylobacter* by direct streak on mCCD agar, but moderately with background flora was present
- Chicken skin samples from the same flock negative for *Campylobacter* by direct streak on mCCD agar and after freeze-storage by enrichment with Bolton and Preston broth
- Packed in zip bags and freeze-stored at –20 °C until distribution
- Three days before distribution: thawed and homogenised with BPW,
  6 ml of caecal suspension aliquoted into plastic tubes,
  stored at 5 °C until distribution of the PT

PT 40: Time to arrival & start of analysis

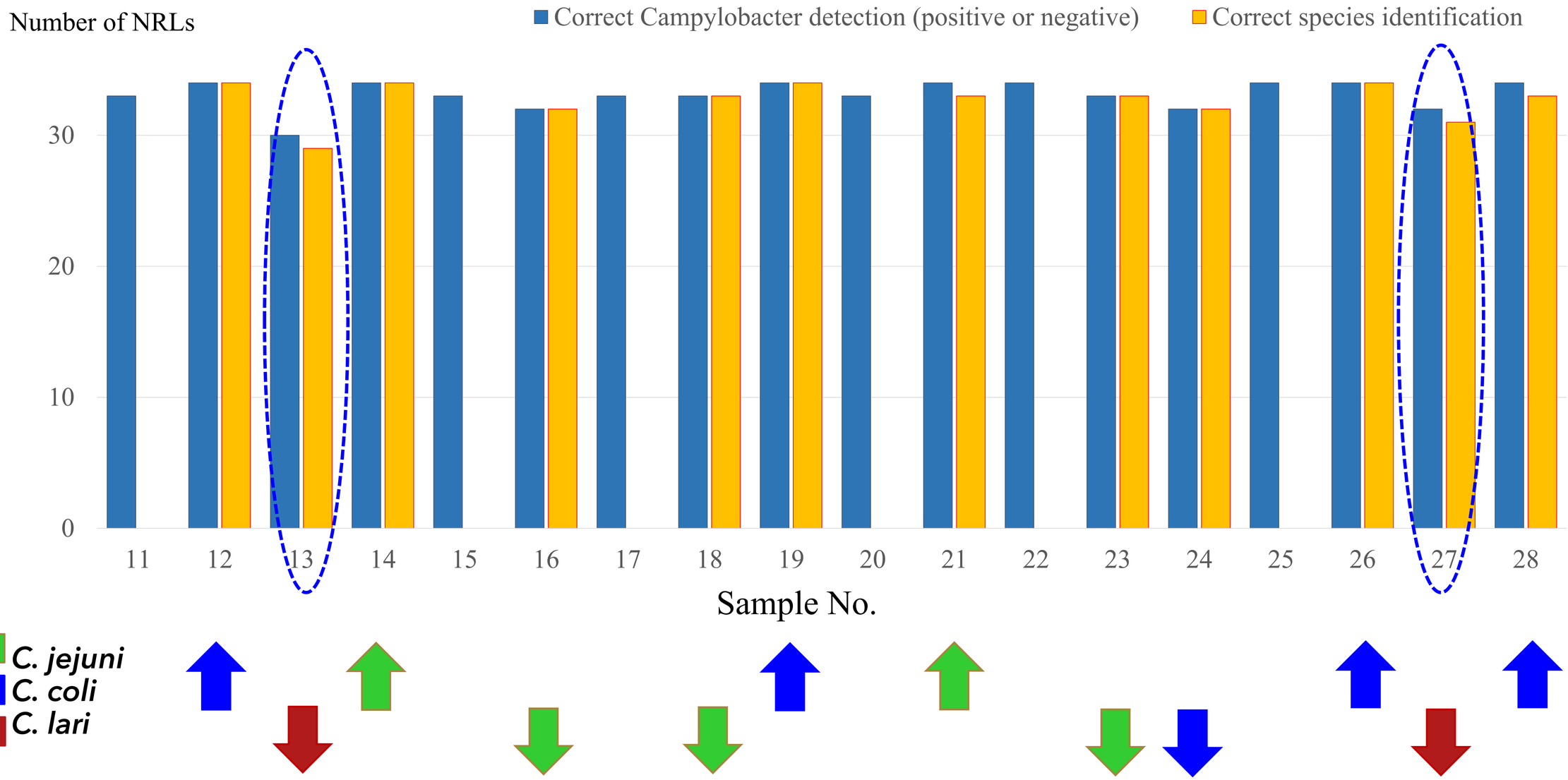


Analysis of the samples included in PT 40 should be started at the latest on the 21st of March 2025.

PT 40: Correct reported results per lab



PT 40: Correct reported results per sample

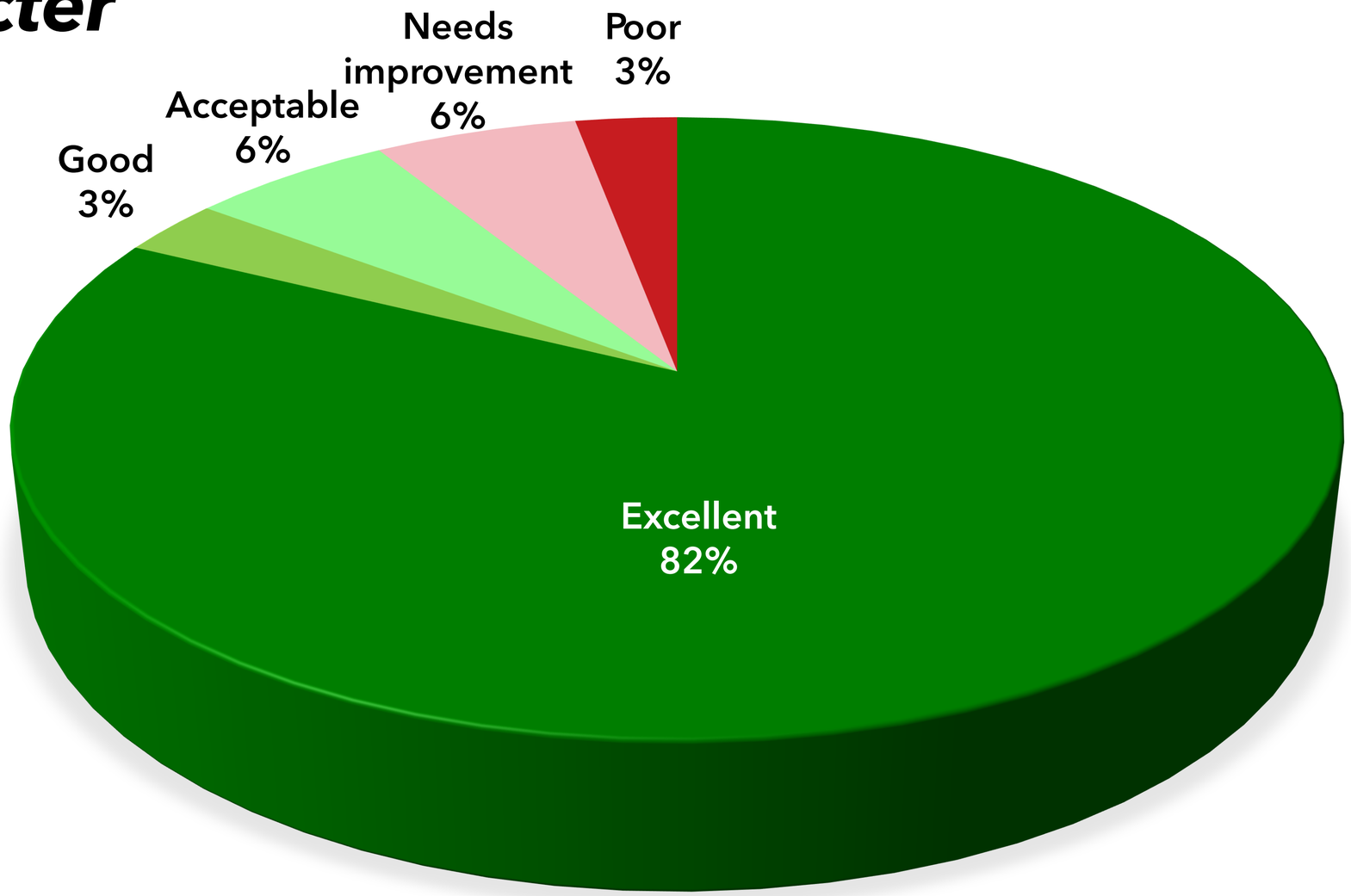


PT 40: Combined performance grades

*Table showing the **minimum number of correct results** needed for each performance grade*

	Category of samples			Measures on the lower limit of each grade					
Performance grade	**Low level**	**High level**	**Neg**	**Se low**	**Se high**	**Se total**	**Sp**	**Acc**	**Se id**
Excellent	6	6	6	100.0%	100.0%	100.0%	100.0%	100.0%	95.0%
Good	5	6	6	83.3%	100.0%	91.7%	100.0%	94.4%	85.0%
Acceptable	4	5	5	66.7%	83.3%	75.0%	83.3%	77.8%	70.0%
Needs improvement	3	4	4	50.0%	66.7%	58.3%	66.7%	61.1%	57.0%

PT 40: Overall performance in detection of *Campylobacter*



PT 40: Species identification

4 misidentifications

2 cases: Sample No. 13 & 27 (*C. lari*) reported as *C. coli*

1 case: Sample No. 21 (*C. jejuni*) reported as *C. coli*

1 case: Sample No. 28 (*C. coli*) reported as *C. lari*

Performance in identification: 32 excellent, no NRL below acceptable limit

PT 40: Educational samples

Sample No.	*Campylobacter* species	Both *C. lari* and *C. upsaliensis*	*C. lari* (but not *C. upsaliensis*)	*C. upsaliensis* (but not *C. lari*)	Both *C. jejuni* and *C. lari*	*C. jejuni* (but not *C. lari*)	*C. jejuni* (in addition)	*C. helveticus* (in addition)	*C. coli*	*Campylobacter* spp. but unable to identify species	No *Campylobacter* detected
29	*Campylobacter lari* *Campylobacter upsaliensis*	**11**	**15**	**1**			2	2	3[a]		1
30	*Campylobacter jejuni* *Campylobacter lari*				**19**	**9**			1[b]	1	

[a] 2 as the only reported species and 1 in addition to *C lari* and *C. upsaliensis*.
[b] As the only reported species.

Thank you for listening!

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