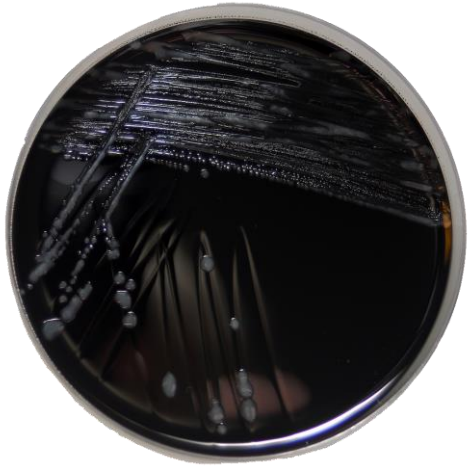
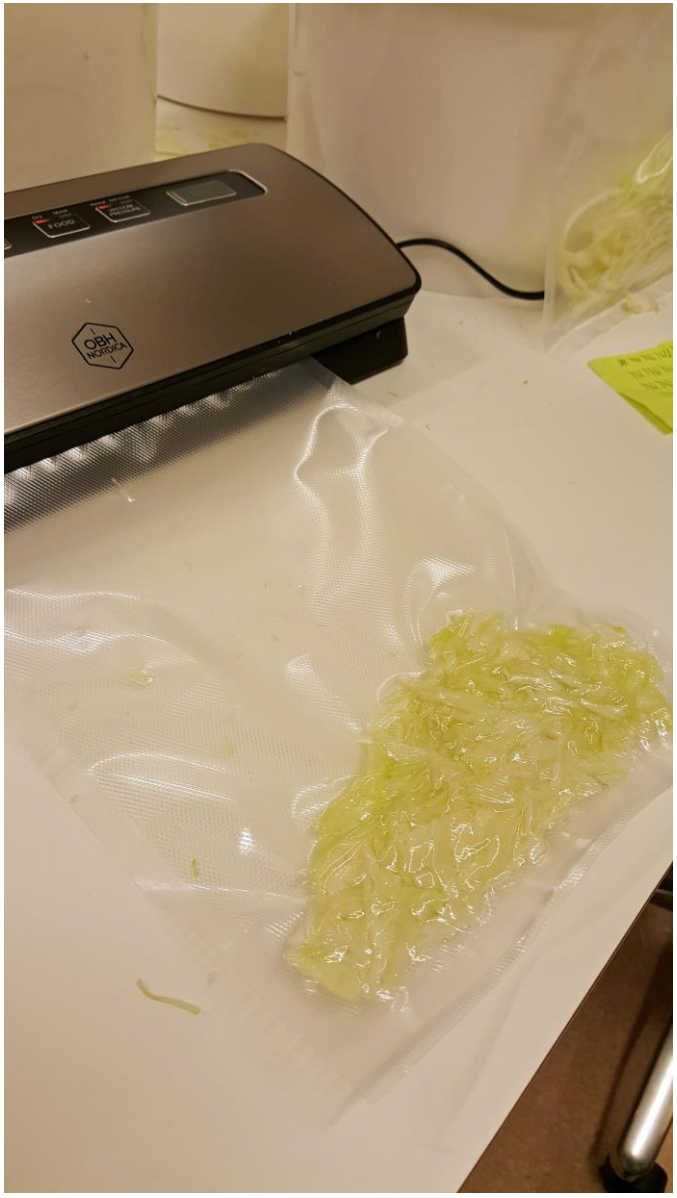
RESULTS OF PROFICIENCY TESTS NO. 29 AND 30

Helena Höök

EURL-*Campylobacter*

Workshop 2021





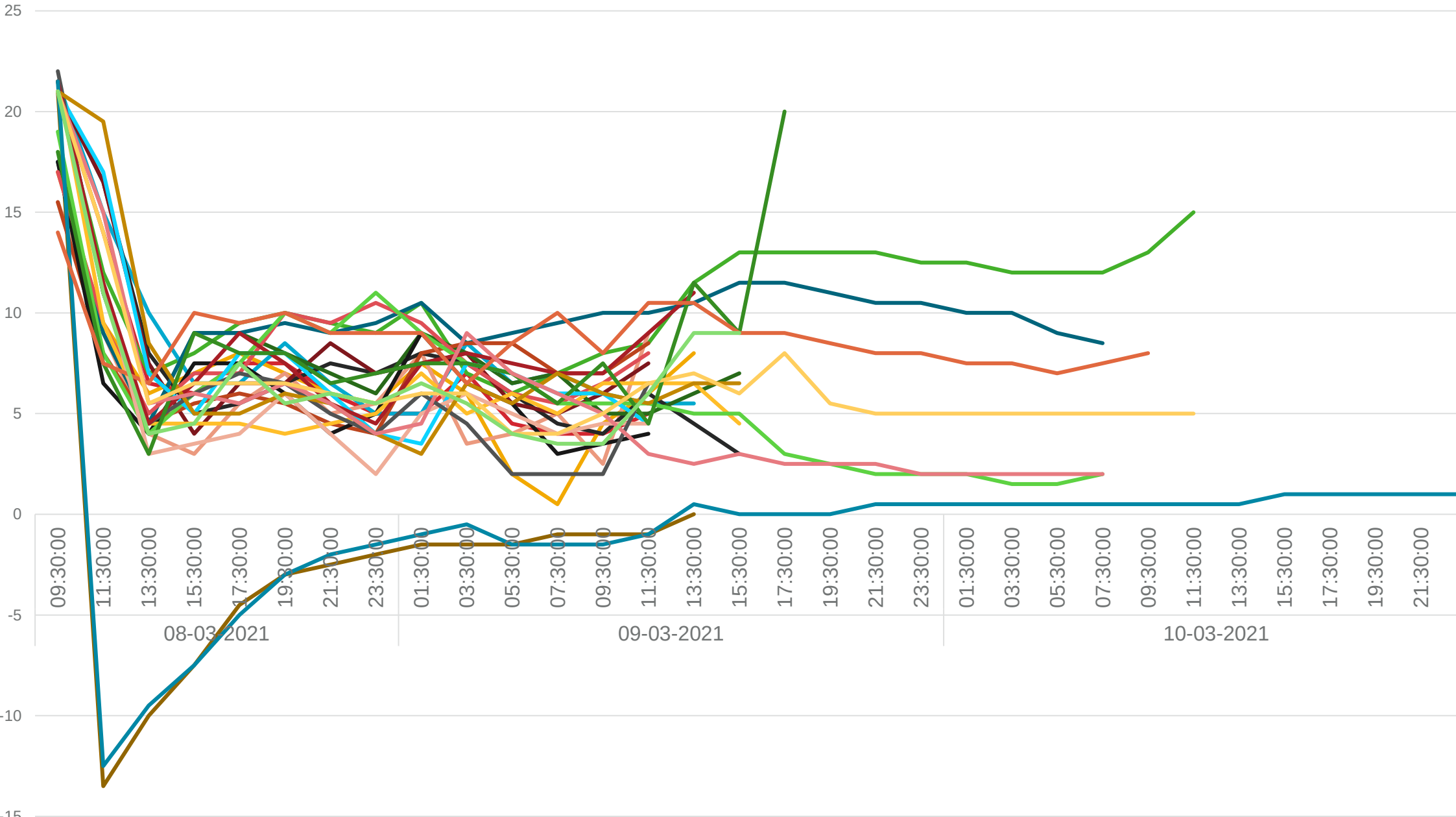
Thank you for your participation and for providing information in the questback reports!

EURL Campylobacter

NUMBERS OF PARTICIPANTS

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

TEMPERATURE DURING TRANSPORT



PT 29 – ENUMERATION (AND SPECIES IDENTIFICATION) IN SHREDDED CABBAGE

PROFICIENCY TEST NO. 29

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

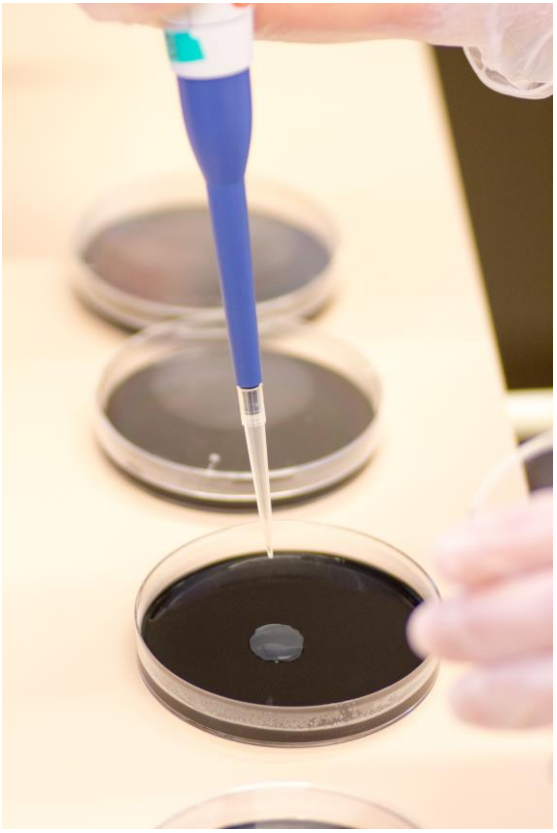
Designed to enable validation of an additional food category to turn the scope of ISO 10272-2 into "broad-range of foods" (voluntary)

- Enumeration and confirmation of *Campylobacter* spp. in shredded cabbage
- 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 29: CONTENTS AND PROCEDURE

- Two bags of shredded cabbage, each of about 60 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.



PT 29: DESCRIPTION OF THE 10 VIALS

Sample No.	Species	Level (log cfu/vial)	Batch No.
1	*C. lari*	5.22	SVA049
2	*C. lari*	4.22	SVA048
3	*C. lari*	6.05	SVA058
4	*C. lari*	6.05	SVA058
5	*C. lari*	4.22	SVA048
6	*C. coli*	4.45	SVA060
7	Negative		
8	*C. lari*	5.22	SVA049
9	*C. jejuni*	4.53	SVA059
10	*Escherichia coli*	4.74	SVA045

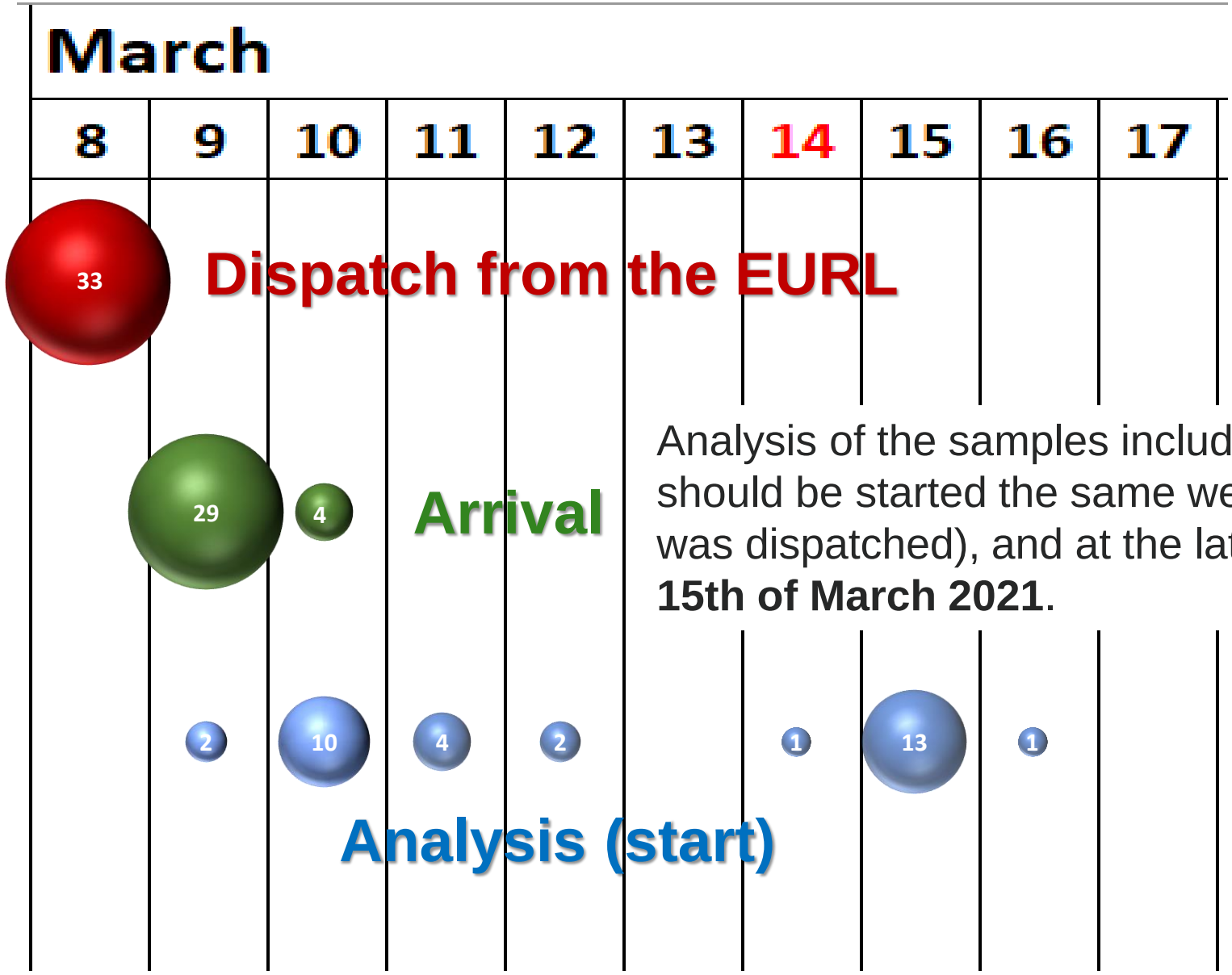
PT 29: QUALITY CONTROL

- Vials produced by the EURL
- Vials tested for homogeneity and stability, and in triplicates on mCCDA
- Enumerations with shredded cabbage for control of *Campylobacter* levels and homogeneity
- Tested four times, once before and three times after dispatch
- Stability during transport conditions should have been tested more thoroughly *before* dispatch …

PT 29: PREPARATION OF THE CABBAGE

- Pre-tests with a first batch of cabbage to test and adapt the preparation process
- Cabbage bought in retail 15 days before distribution
- Tested negative for presence of *Campylobacter*
- Tested for background flora
- Shredded and divided into portions of about 60 g
- Vacuum-packed in plastic bags 14 days before dispatch
- Stored at −4 °C until distribution

PT 29: TIME TO ARRIVAL & START OF ANALYSIS



Analysis of the samples included in PT 29 should be started the same week as the test was dispatched), and at the latest on the **15th of March 2021**.

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%

HOW WAS PERFORMANCE CALCULATED?

Adaptions because of use of duplicates and low levels

- Duplicate vials (2 and 5, 1 and 8, 3 and 4)
  - 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
- −2σMADe and/or -3σMADe limits below 1.0 log cfu/g
  - minimum score given for results below this level adjusted
  - included results where no campylobacters were reported

Grade	Scoring limits	
Excellent	20	95.1–100%
Good	17–19	85.0–95.0%
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Needs improvement	12–13	57.0–69.9%
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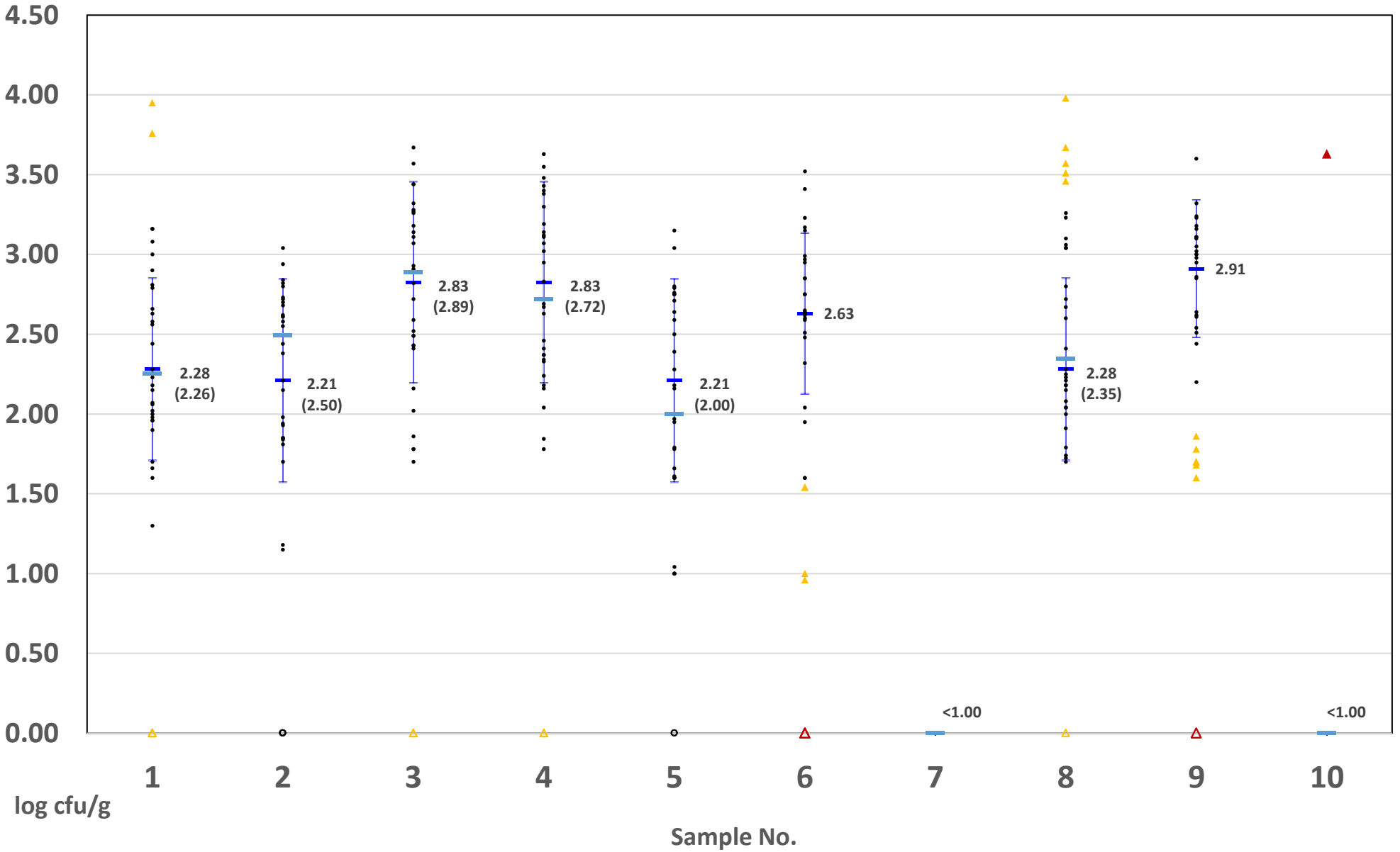
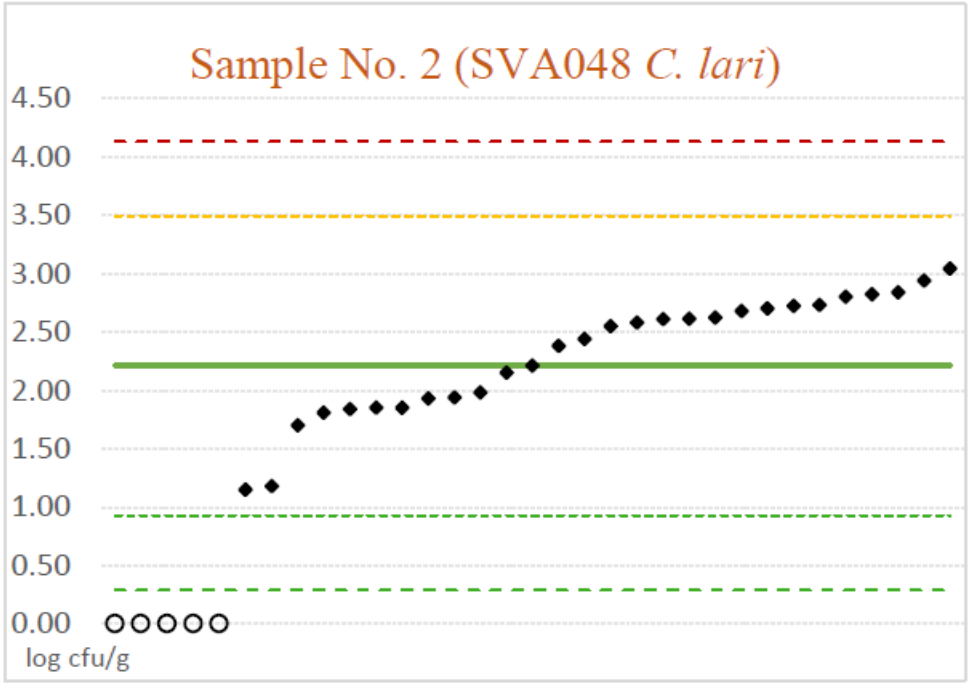
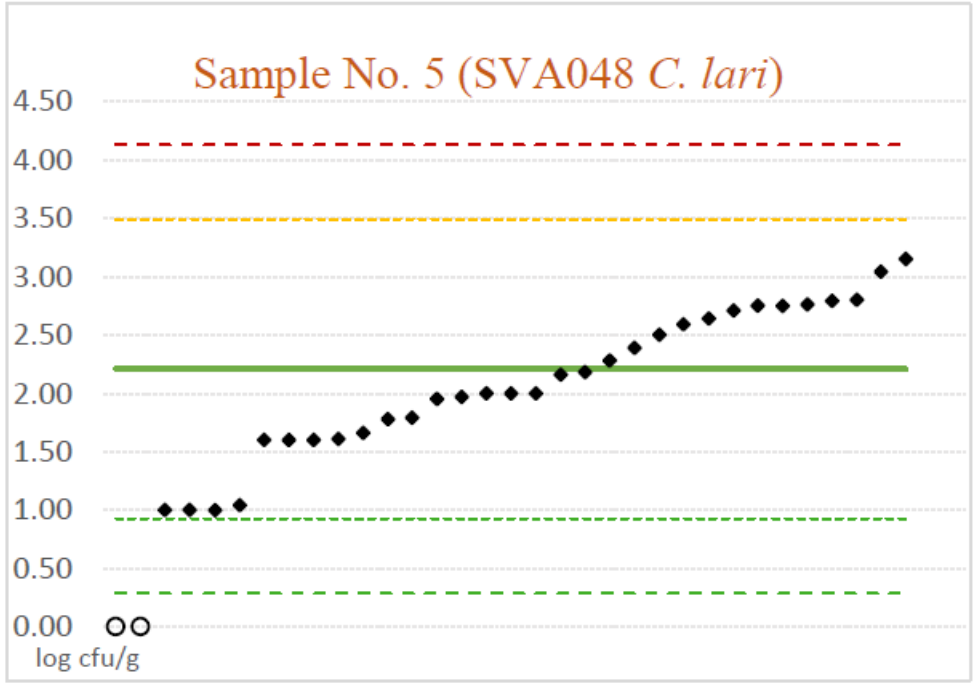
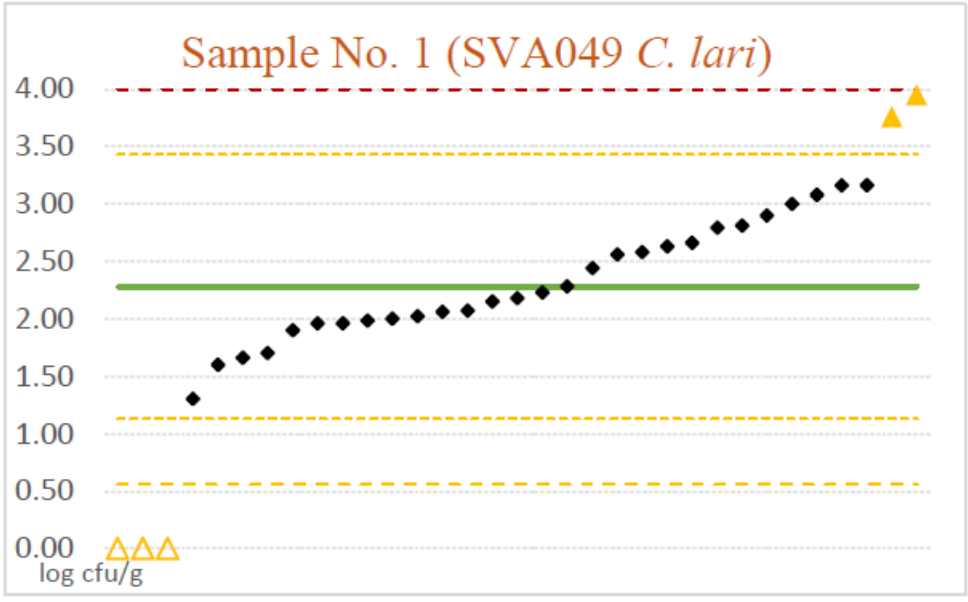
PT 29: RESULTS OF ENUMERATION

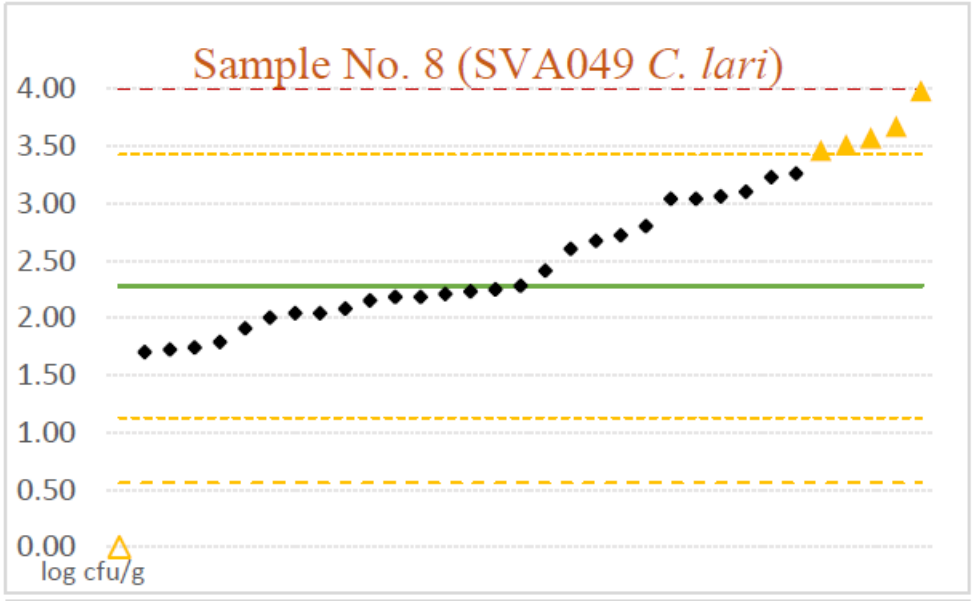


PT 29: RESULTS OF ENUMERATION

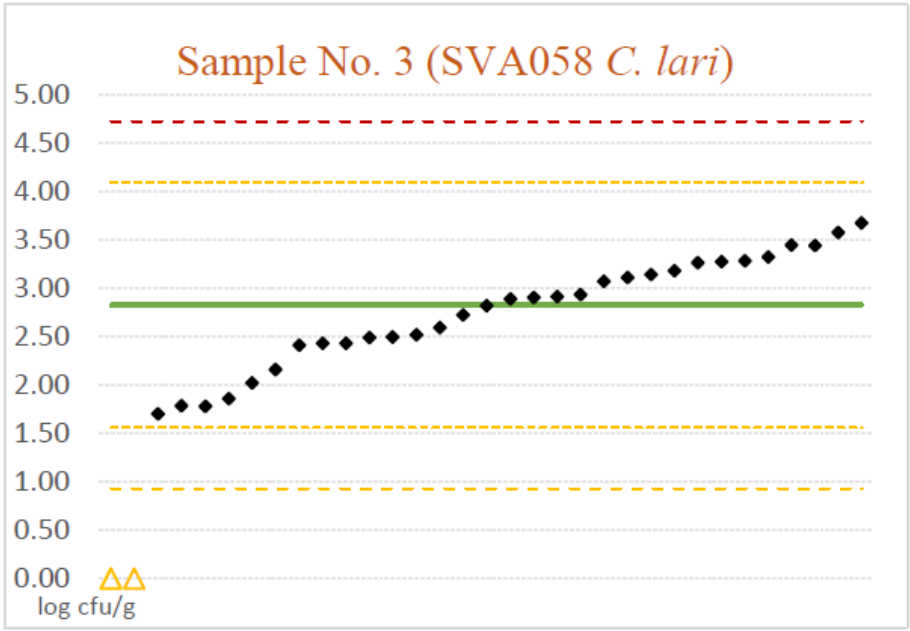


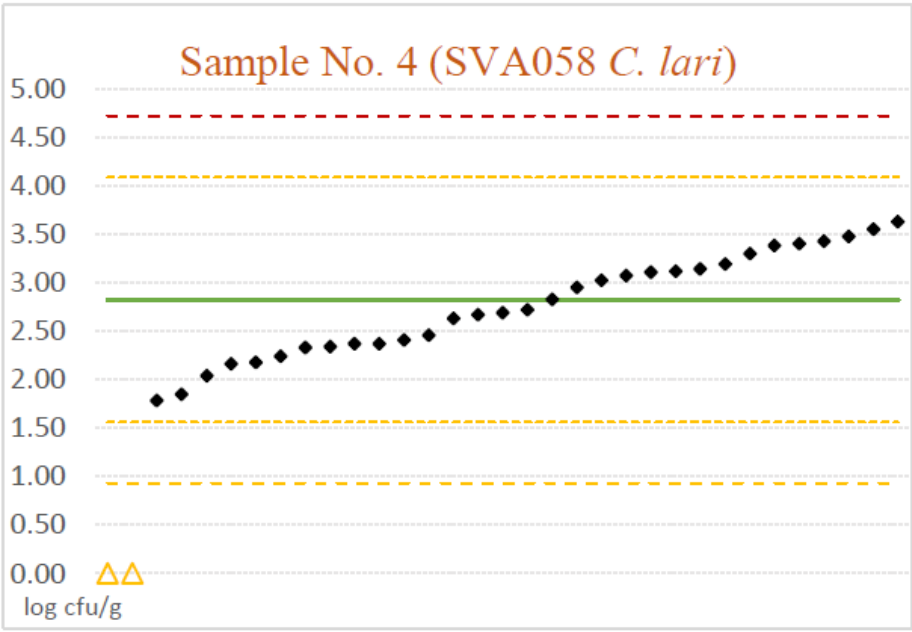


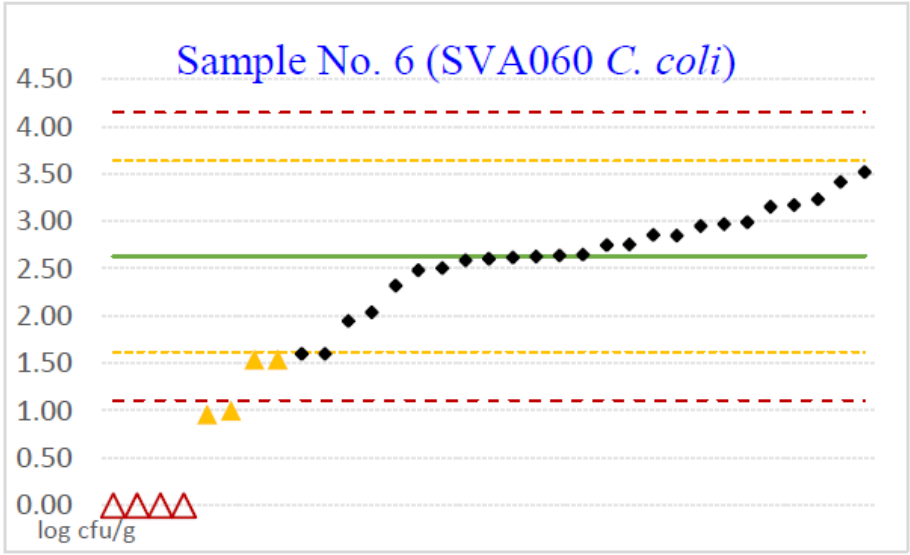


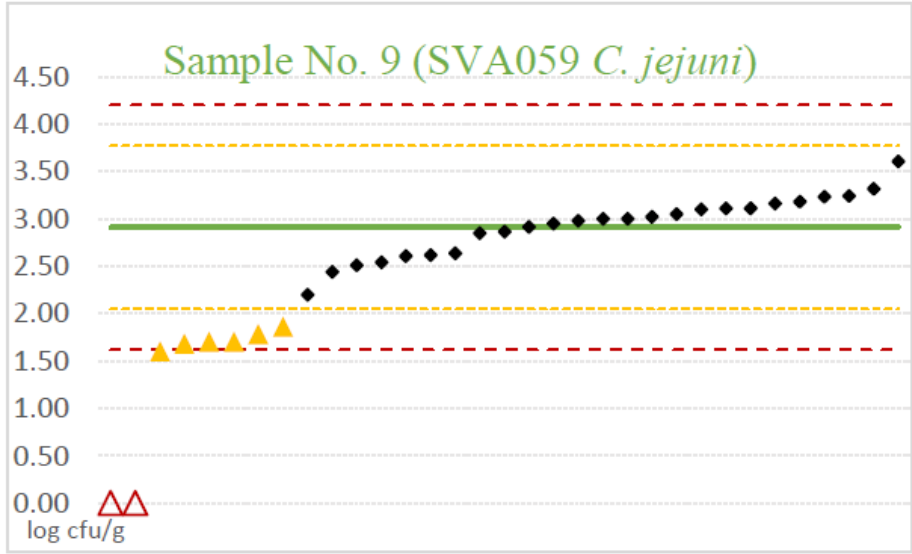


PT 29: RESULTS OF ENUMERATION



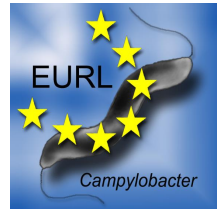




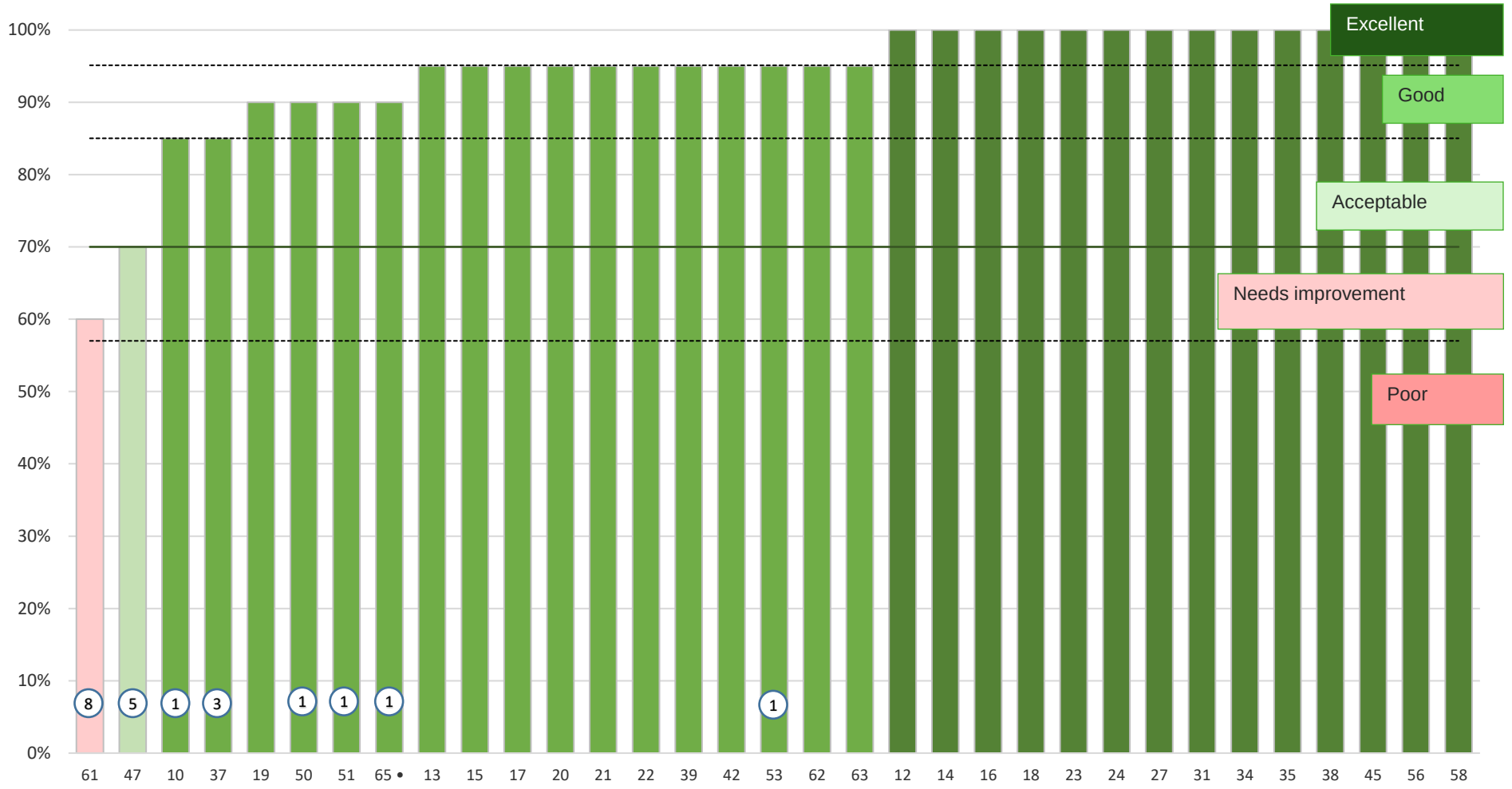


VARIABILITY IN PT ENUMERATION RESULTS

		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
	mean	3.69	1.61	2.50	2.46	0.37	0.20	0.29	0.28



PERFORMANCE PT 29



PT 29: SPECIES IDENTIFICATION (VOLUNTARY)

Content of sample (vial)	*C. jejuni*	*C. coli*	*C. lari*	No growth	Growth of other
1. ***C. lari***			**27**	2	
2. ***C. lari***			**27**	2	
3. ***C. lari***			**28**	1	
4. ***C. lari***			**28**	1	
5. ***C. lari***			**28**	1	
6. ***C. coli***		**26**	**1**		
7. Negative				**26**	**3**
8. ***C. lari***			**29**		
9. ***C. jejuni***	**26**				
10. *E. coli*				**4**	**25**

PT 30 – DETECTION AND SPECIES IDENTIFICATION OF *CAMPYLOBACTER*

EURL Campylobacter

PROFICIENCY TEST NO. 30

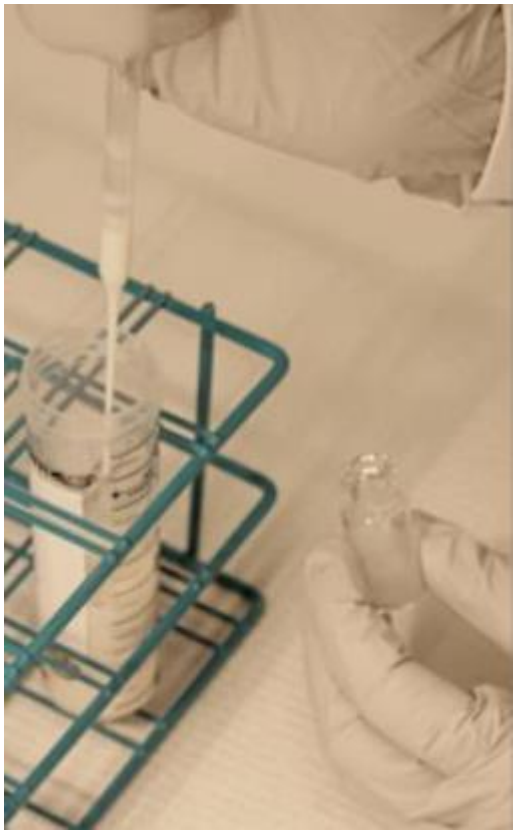
The objective was to assess the performance of the NRLs to detect and identify *Campylobacter* species in raw milk.

- Detection of *Campylobacter* spp. in raw milk (food or animal samples)
- Species identification of *Campylobacter*
- 18 samples: 6 low level, 6 high level, 6 negative
- Recommended method ISO 10272-1:2017, but other methods allowed
- Enrichment in Bolton broth (procedure A in ISO 10272-1) was recommended in first hand
- Enough milk provided for performing two enrichment procedures in parallel (if of interest for the laboratory)

PT 30: CONTENTS AND PROCEDURE: RAW MILK

- A bottle with about 420 ml of raw milk
- 18 freeze-dried vials (with or without *Campylobacter* and/or other bacteria)
- Mix each vial with raw milk up to a total volume of 22 ml (allowing up to two test portions of 10 ml)
- Follow the method(s) of choice for
  - detection
  - species identification

of *Campylobacter* spp.



DESCRIPTION OF THE 18 VIALS IN PT 30

Sample No.	Content in vial	Batch No.	Level	log cfu/vial	log cfu/10 ml	× LOD_{50}
11	Negative					
12	***C. lari***	SLV300	Low	2.81	2.47	5
13	***C. jejuni***	SVA025	Low	3.20	2.86	13
14	***C. jejuni***	SVA059	High	4.53	4.19	272
15	*E. coli*	SVA045		4.74		
16	Negative					
17	***C. coli***	SVA060	High	4.45	4.11	226
18	***C. lari***	SVA050	High	3.95	3.61	71
19	***C. lari***	SVA048	High	4.22	3.88	133
20	***C. lari***	SVA054	Low	3.14	2.80	11
21	***C. jejuni***	SVA059	High	4.53	4.19	272
22	***C. coli***	SVA051	Low	3.19	2.85	12
23	***C. jejuni***	SVA055	Low	3.28	2.94	15
24	*E. coli*	SVA045		4.74		
25	*E. coli*	SVA045		4.74		
26	***C. coli***	SVA060	High	4.45	4.11	226
27	Negative					
28	***C. coli***	SVA051	Low	3.19	2.85	12

PT 30: QUALITY CONTROL

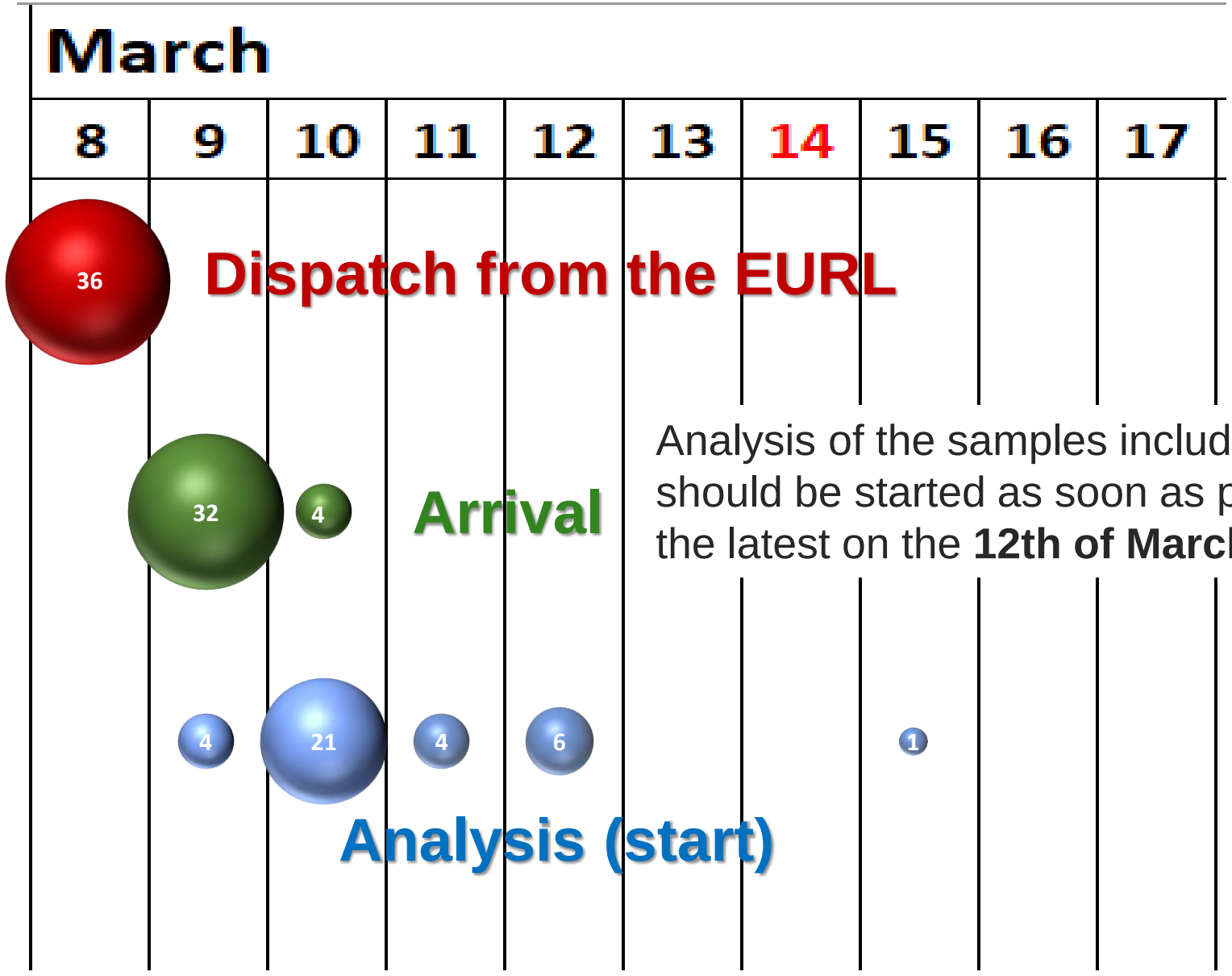
- Vials produced by EURL or the Swedish Food Agency
- Tested for homogeneity and stability by the producer

- Vials tested on mCCDA and with milk before included in the PT
- Vials together with matrix were analysed according to ISO 10272-1:2017, procedure A and B
- Tested three times, once before and twice after dispatch

PT 30: PREPARATION OF THE RAW MILK

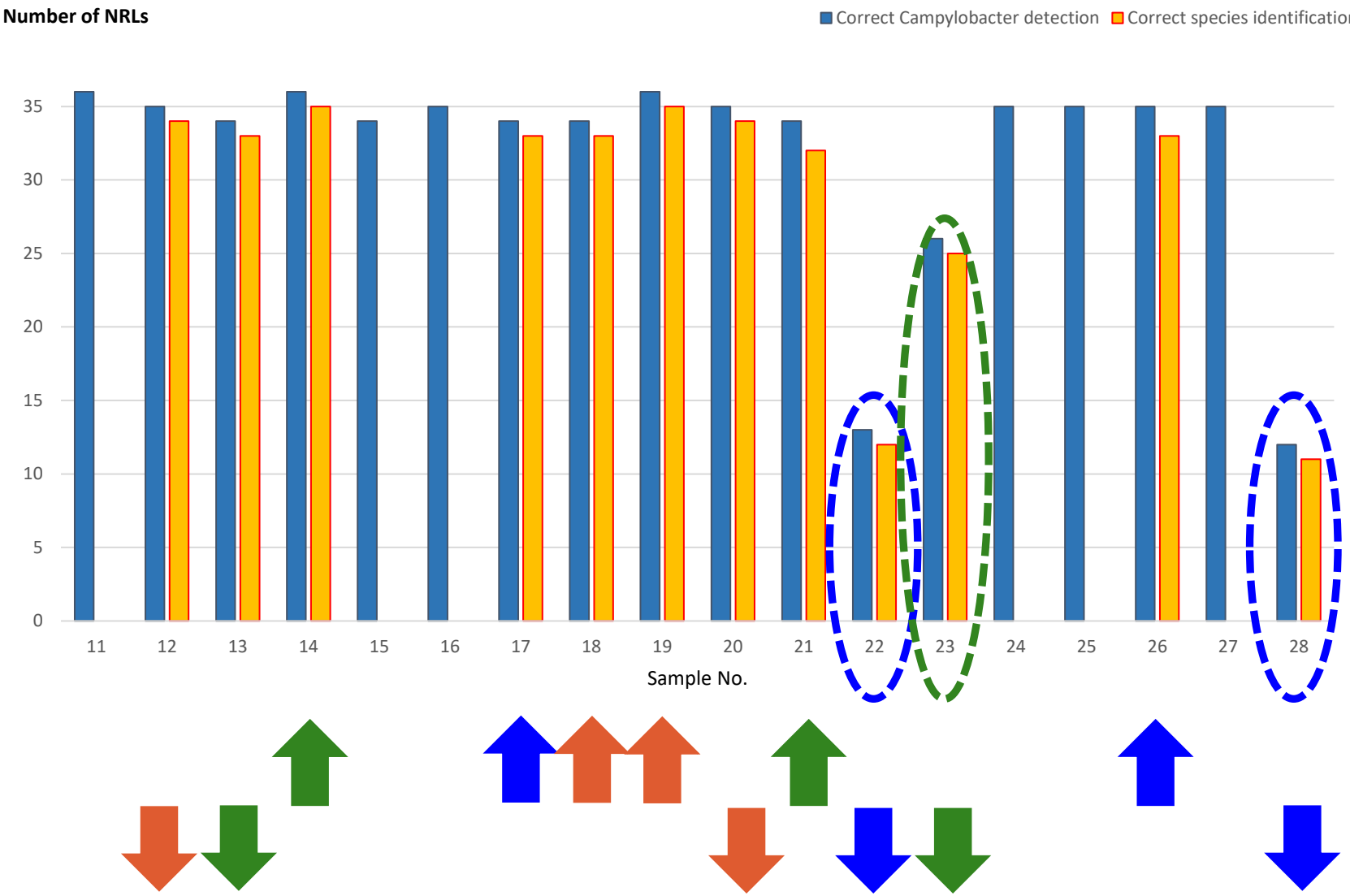
- Obtained directly from a milk farm two months before the PT
- Tested negative for presence of *Campylobacter* spp.
- Tested for background flora
- Stored at −20 °C until distribution

PT 30: TIME TO ARRIVAL & START OF ANALYSIS

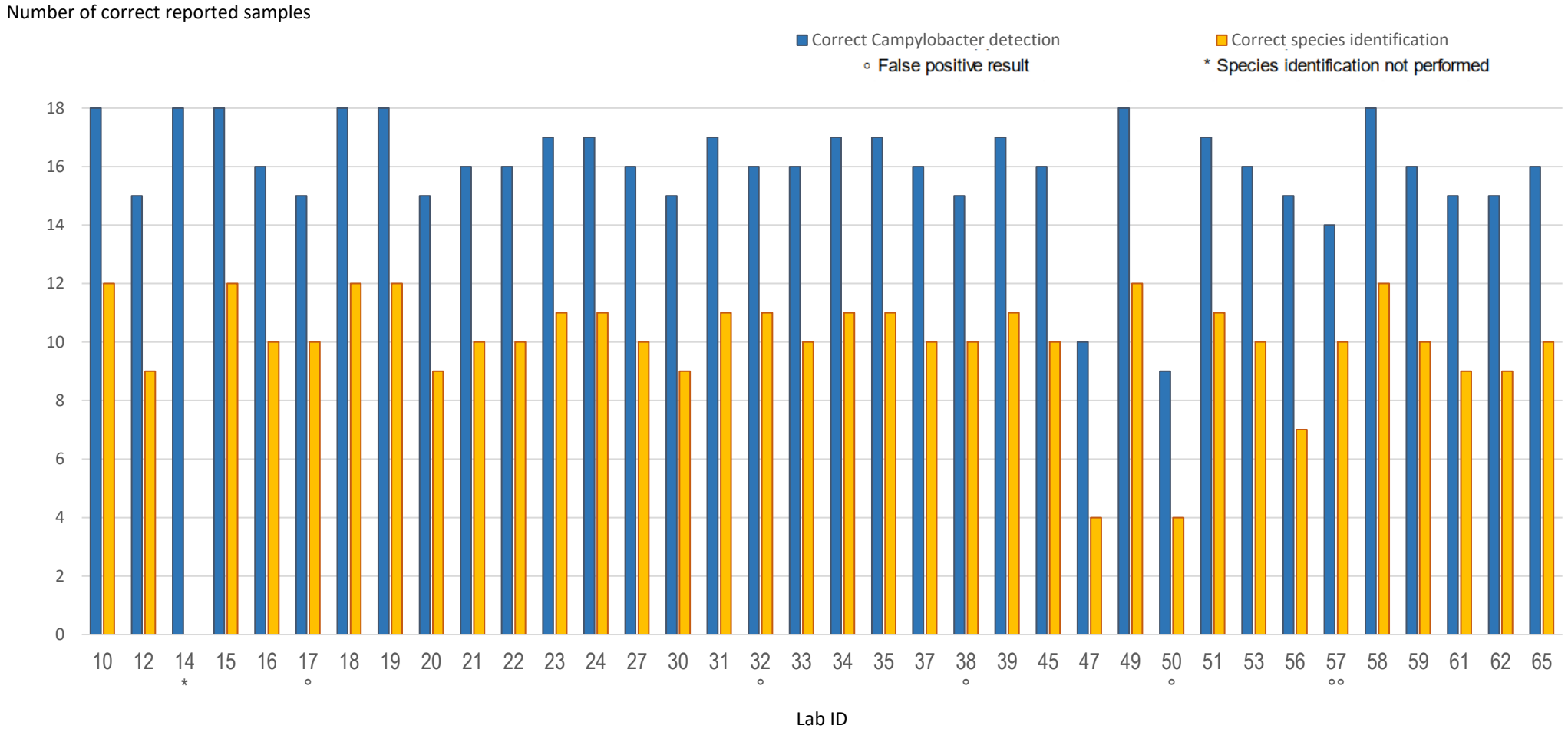


Analysis of the samples included in PT 30 should be started as soon as possible and at the latest on the **12th of March 2021**.

PT 30: CORRECT REPORTED RESULTS PER SAMPLE



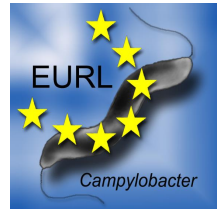
PT 30: CORRECT REPORTED RESULTS PER LAB



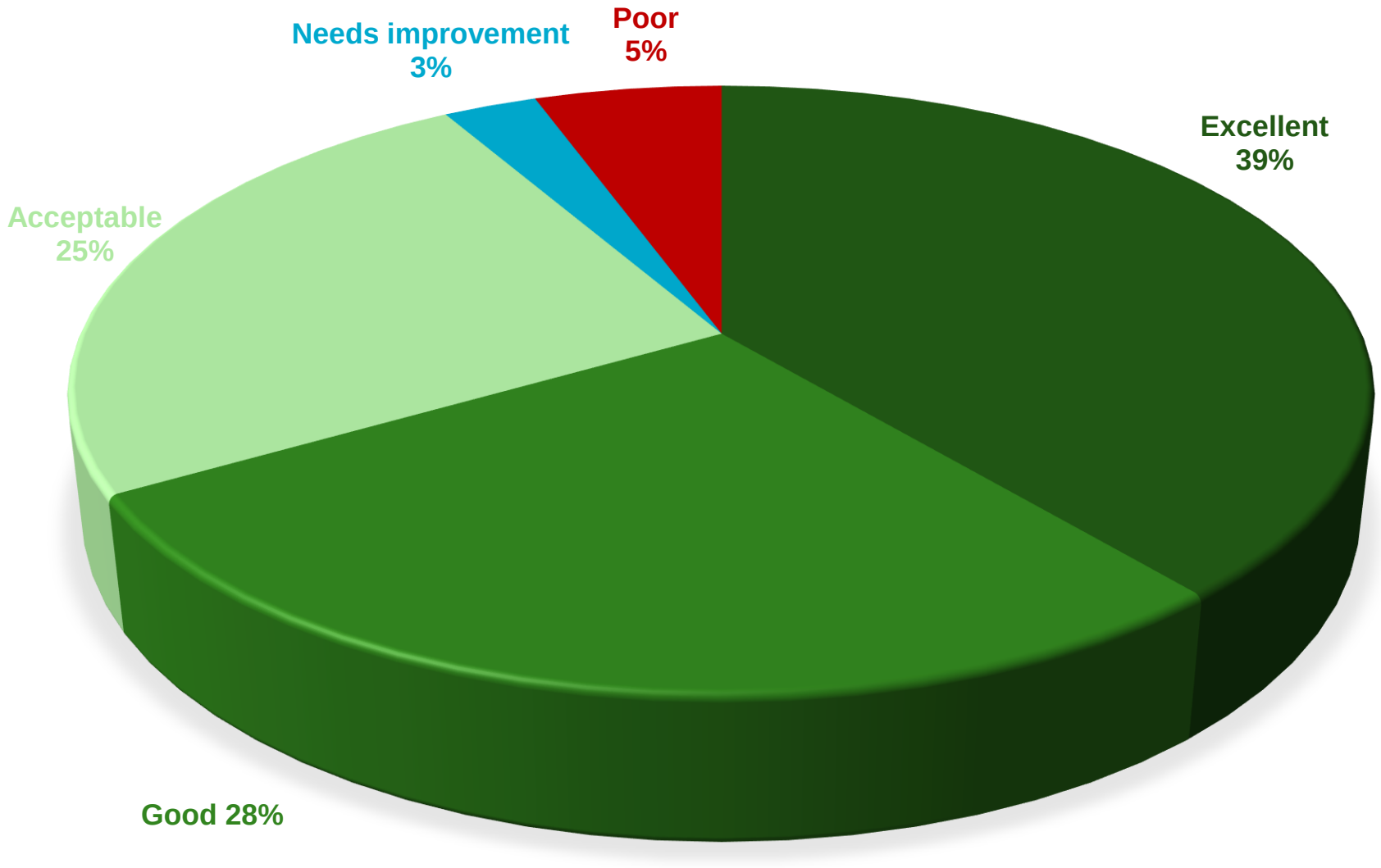
PT 30: COMBINED PERFORMANCE GRADE

*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**	**Acc**	**Sp**	**Se id**
Excellent	5	6	6	83%	100%	92%	94%	100%	95%
Good	4	5	6	67%	83%	75%	83%	100%	85%
Acceptable	3	4	5	50%	67%	58%	67%	83%	70%
Needs improvement	2	3	4	33%	50%	42%	50%	67%	57%

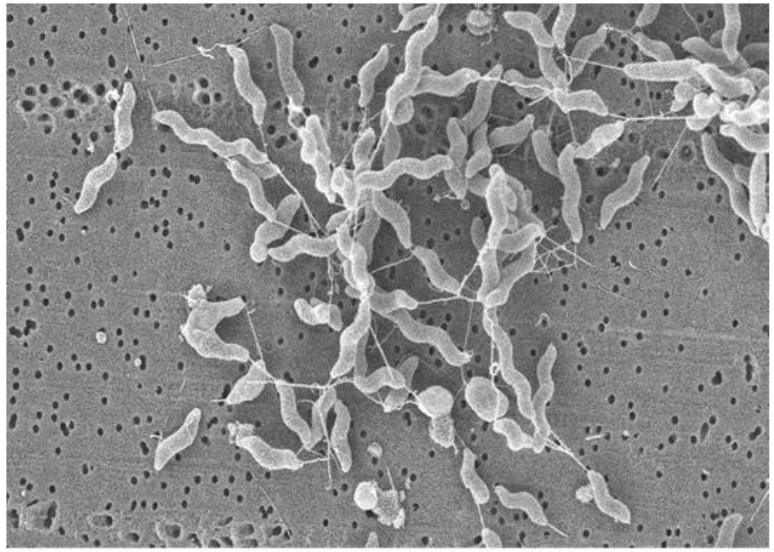


PT 30: OVERALL PERFORMANCE IN DETECTION OF *CAMPYLOBACTER*



PT 30: SPECIES IDENTIFICATION

- Only two misidentifications (by the same laboratory)
  - Sample No. 21 (*C. jejuni*) reported as *C. lari*
  - Sample No. 26 (*C. coli*) reported as *C. jejuni*



EURL Campylobacter

THANK YOU FOR LISTENING!

EURL Campylobacter