RESULTS OF PROFICIENCY TESTS NO. 21 AND 22
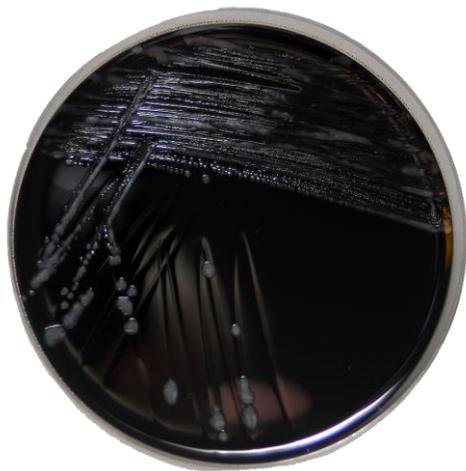
Helena Höök

EURL-*Campylobacter*

Workshop 2018

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

SVA

NUMBERS OF PARTICIPANTS

Year	2018	2017	2016	2015	2014	2013	2012	2011	2010
	PT 21	PT 19	PT 17	PT 15	PT 13	PT 11	PT 9	PT 8	PT 7
Enumeration	37	36	36	36	35	36	33	33	31
	PT 22	PT 20	PT 18	PT 16	PT 14	PT 12	PT 9	PT 8	PT 7
Detection & species id	31	34	33	32	36	34	36	34	34

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CAMPYLOBACTER-FREE MATRICES

- Chicken skin (PT 21) & caeca (PT 22) from a producer with no *Campylobacter*-positive broiler flocks for >1 year

- Slaughterhouse with very low level of *Campylobacter-positive* flocks

  - 3,7 % during 2017

  - 0 % Dec 2017 – Mar 2018

- Skin and ceacal material tested negative for presence of *Campylobacter*

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TEMPERATURE DURING TRANSPORT

Temperature Micro-T-logs PT 21 and 22, 2018
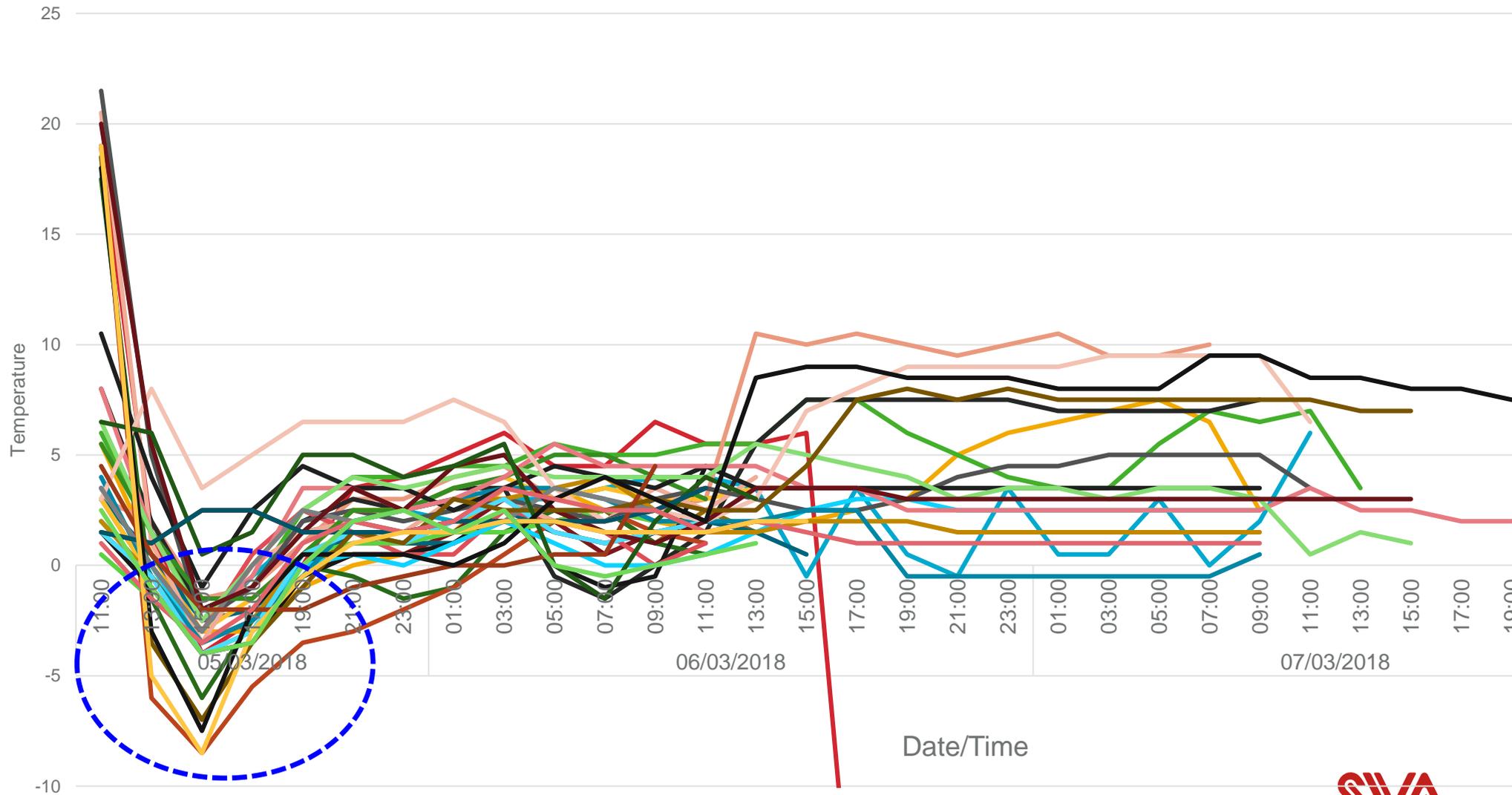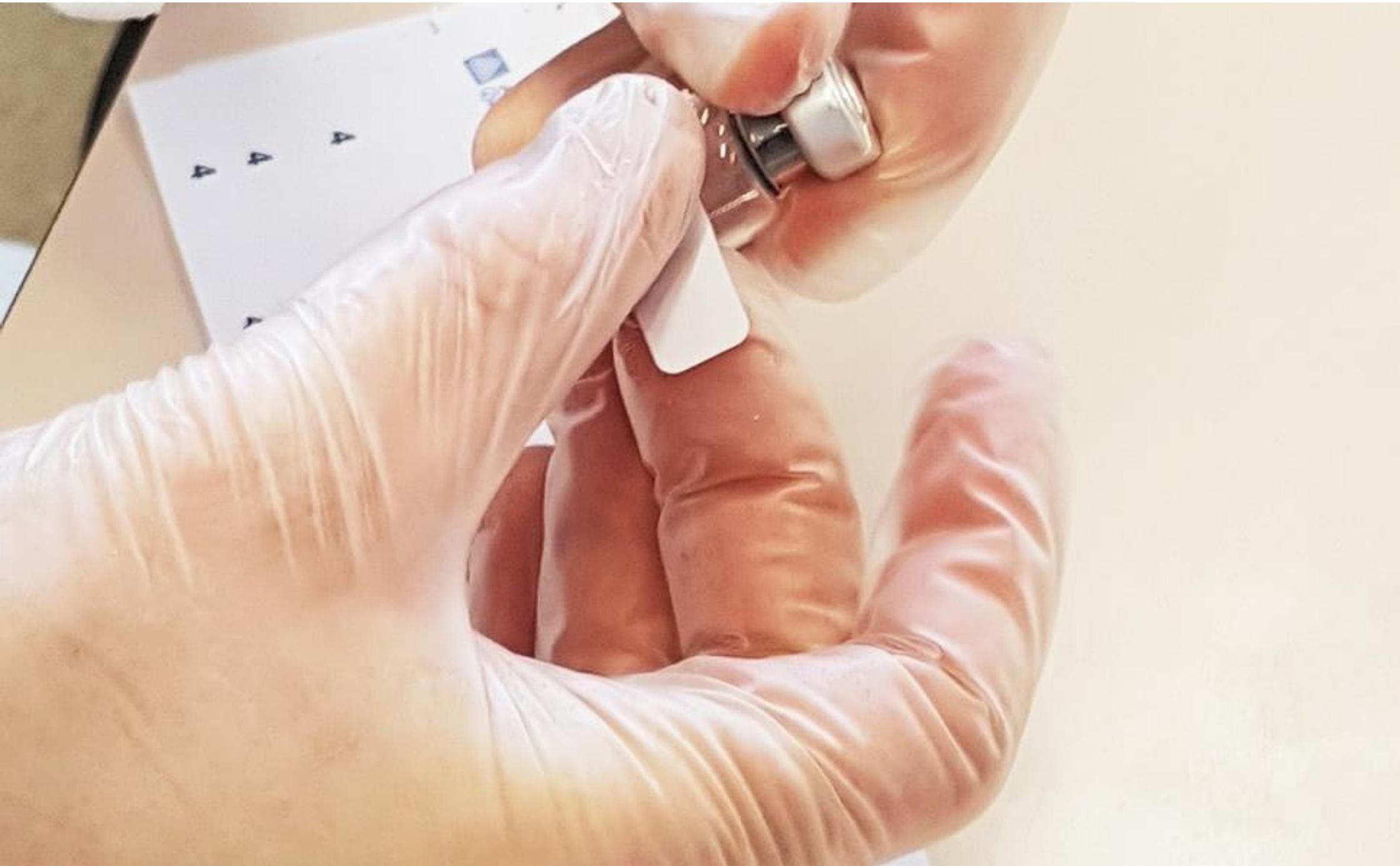
PROFICIENCY TEST NO. 21

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

- Enumeration (quantification) and confirmation of *Campylobacter* spp. in chicken skin

- Detection of *Campylobacter* spp. in chicken skin (voluntary)

- Species identification of *Campylobacter* (voluntary)

- Recommended method ISO 10272:2017, but other methods allowed

- Should allow enumeration of between 10 and 10^5 cfu *Campylobacter*/g chicken skin

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PT 21: CONTENTS AND PROCEDURE

- Chicken skin (110–120 g) to be divided into 10 portions of 10 g

- 10 vials with freeze-dried sample (with or without *Campylobacter*)

- Homogenize and make a initial dilution of 10^{-1}

- Follow the method(s) of choice for

  - enumeration

  - detection (voluntary)

  - species identification (voluntary)

of *Campylobacter* spp.

DESCRIPTION OF THE 10 VIALS IN PT 21

Sample No.	Species	Batch No.
1	Negative	151
2	*Campylobacter lari*	248
3	*Campylobacter lari*	299
4	*Escherichia coli*	150
5	*Campylobacter coli* + *Escherichia coli*	221
6	*Campylobacter jejuni*	235
7	*Campylobacter coli*	SVA007
8	*Campylobacter jejuni*	SVA004
9	*Campylobacter jejuni*	SVA010
10	*Campylobacter jejuni*	259

PT 21: QUALITY CONTROL

- Vials produced by EURL (7, 8, 9) or the National Food Agency

- Tested for homogeneity and stability by the producer

- Enumerations with chicken skin in triplicates for control of *Campylobacter* levels and homogeneity

- Maximum difference allowed: 0.50 log cfu/g

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PT 21: TIME TO ARRIVAL & START OF ANALYSIS
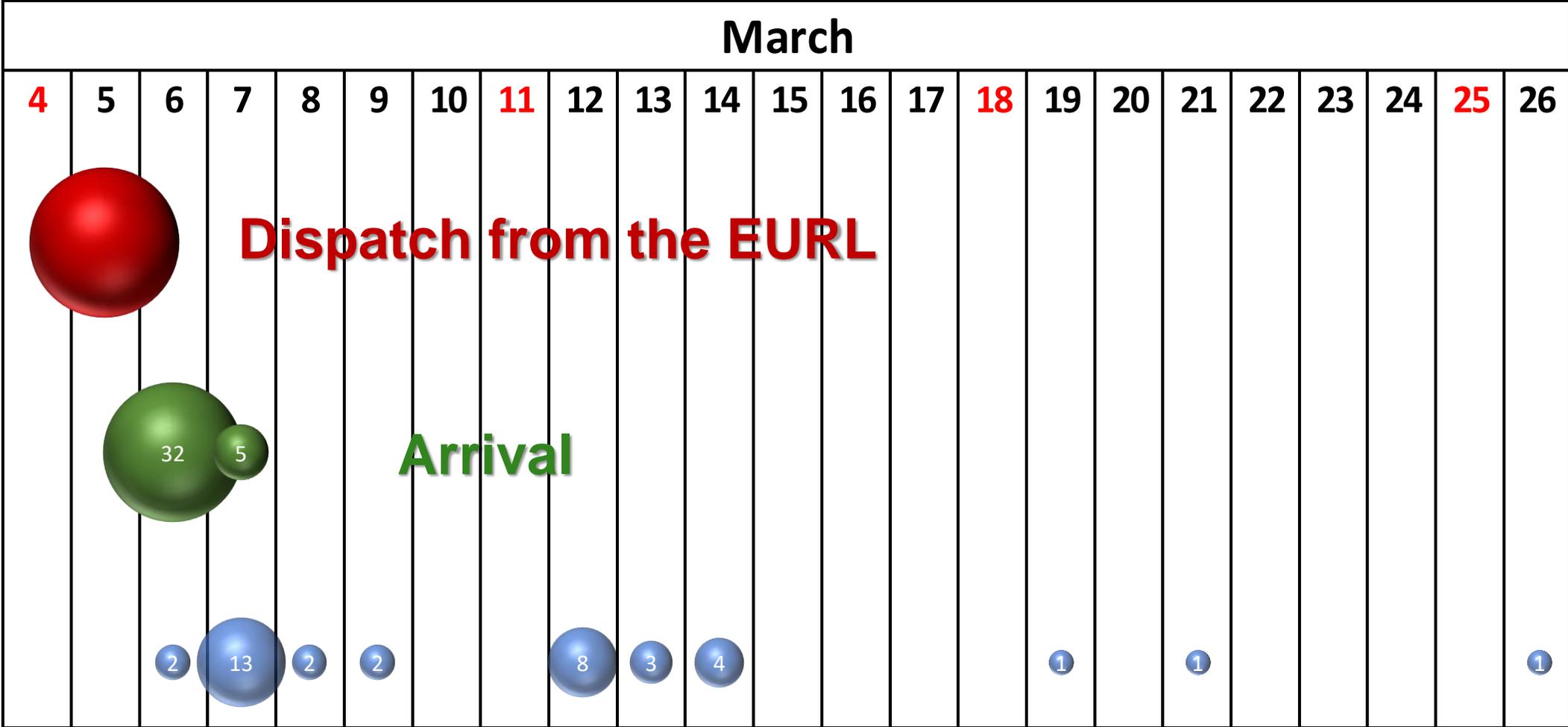
PT 21: METHODS

Reported method for enumeration	No. of NRLs
ISO 10272:2017	31
ISO 10272:2006	2
NMKL 119, 3rd ed. 2007	2
Other methods	2

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WHAT'S IN THE RESULTS?

- Laboratory procedures
  - Dilution
  - Spreading
  - Counting
  - Confirmation
- Calculations
- Reporting
- Final results
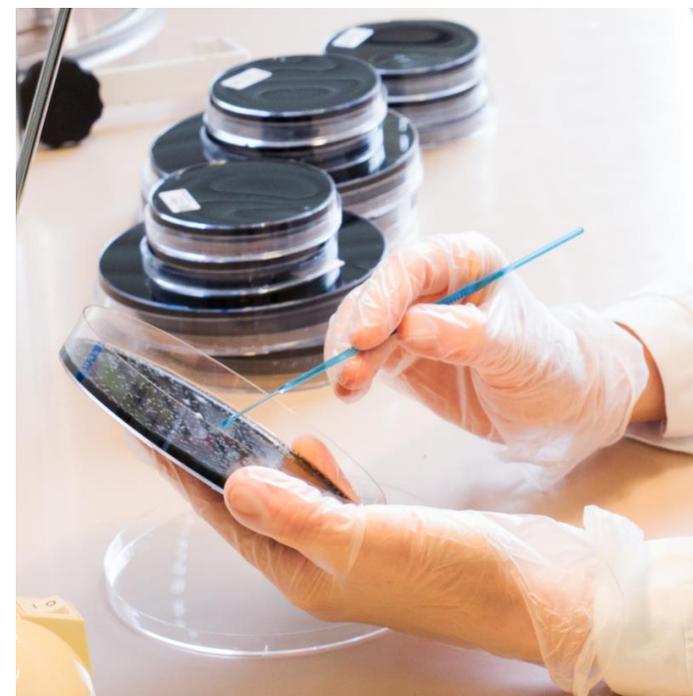
Please fill in the results of the enumeration of *Campylobacter* spp. in log cfu/g. If no *Campylobacter* have been found, report the result as lower than the detection limit, e.g. lower than (less-than sign) 1.0.

PLEASE NOTE: The given results will be considered as final answers and will be used in the calculation of performance.

12) **Final results of enumeration in log cfu/g**

* 1. <1.00

* 2. 2.34

* 3. 3.45

Results EURL-*Campylobacter* Proficiency Test Number 21 2018

Enumeration (and voluntary detection and species identification) of *Campylobacter* in chicken skin

	Score	Performance
Overall enumeration	**75.0%**	**Acceptable**
Sensitivity detection (voluntary)	**100.0%**	**Excellent**
Sensitivity identification (voluntary)	**87.5%**	**Good**

Country Testland
Laboratory The laboratory of food

NRL lab ID	Name of contact person	Date of arrival	Analysis start
100	Test Testsson	3/6/2018	3/6/2018

	Sample 1.	Sample 2.	Sample 3.	Sample 4.	Sample 5.	Sample 6.	Sample 7.	Sample 8.	Sample 9.	Sample 10.
Contents	Negative	*Campylobacter lari*	*Campylobacter lari*	*Escherichia coli*	*Campylobacter coli* / *Escherichia coli*	*Campylobacter jejuni*	*Campylobacter coli*	*Campylobacter jejuni*	*Campylobacter jejuni*	*Campylobacter jejuni*
Batch No.	151	248	299	150	221	235	SVA007	SVA004	SVA010	259

Enumeration of *Campylobacter* spp. (mandatory)

value below median value −2.58σMAD / z-score below −2	value between median value −2σMAD and −2.58σMAD
value above median value +2.58σMAD / z-score above 2	value between median value +2σMAD and +2.58σMAD
false positive	

	Sample 1.	Sample 2.	Sample 3.	Sample 4.	Sample 5.	Sample 6.	Sample 7.	Sample 8.	Sample 9.	Sample 10.
Lab's results enumeration (log cfu/g)	<1.00	3.60	2.34	2.34	2.23	4.40	4.10	3.50	3.80	2.93
Results as reported	0	3.6	2.335	2.34	2.23	4.4	4.1	3.5	3.8	2.93
Score (points)	2	2	1	0	2	2	2	2	2	0
Z-score	–	-0.40	-1.58	–	-0.47	0.32	-0.13	0.41	0.27	3.03
Median	<1.00	3.90	3.10	<1.00	2.50	4.38	4.30	3.20	3.74	1.98
MAD	–	0.29	0.22	–	0.33	0.47	0.49	0.27	0.19	0.17
σMAD	–	0.43	0.33	–	0.49	0.70	0.73	0.40	0.28	0.25
Mean	–	3.82	3.03	–	2.53	4.16	4.22	3.21	3.65	1.97
SD	–	0.56	0.44	–	0.65	0.75	0.91	0.70	0.54	0.32

Detection and species identification of *Campylobacter* spp. (voluntary)

false negative	false positive	incorrect/no species identification

	Sample 1.	Sample 2.	Sample 3.	Sample 4.	Sample 5.	Sample 6.	Sample 7.	Sample 8.	Sample 9.	Sample 10.
Lab's results detection	not detected	detected	detected	not detected	detected	detected	detected	detected	detected	detected
Lab's results species identification	No growth at all	*Campylobacter lari*	*Campylobacter lari*	Growth of other, not Campylobacter	*Campylobacter coli*	*Campylobacter jejuni*	*Campylobacter coli*	*Campylobacter jejuni*	*Campylobacter jejuni*	*Campylobacter coli*

PT 21: RESULTS OF ENUMERATION
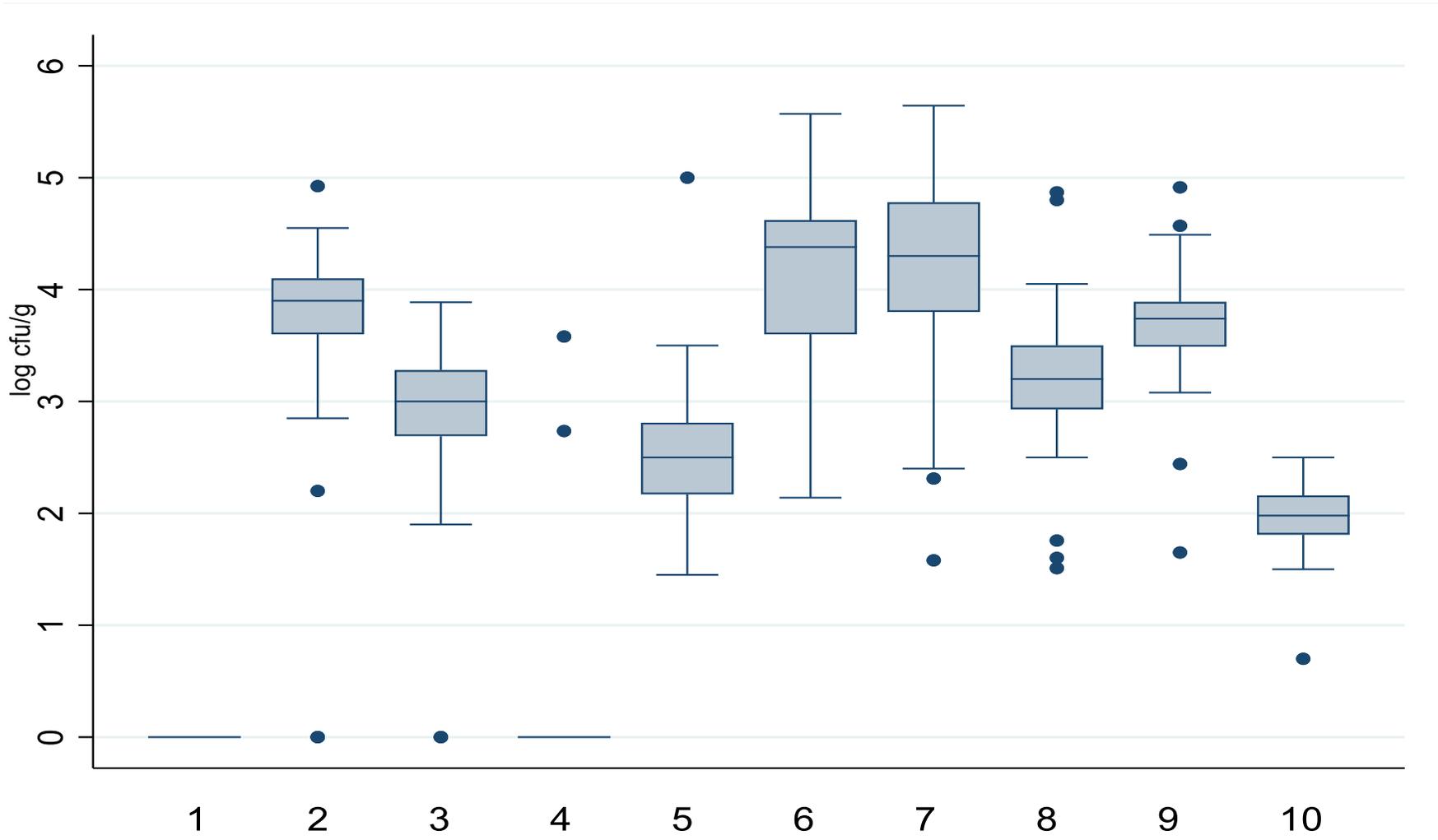
HOW WAS PERFORMANCE CALCULATED?

- The Median Absolute Deviation (MAD) to calculate performance

- σMAD = MAD × 1.4826

- *Campylobacter*-containing samples
  - Results within participants' median ±2σMAD = 2 points
  - Results between ±2σMAD and ±2,58σMAD = 1 point
  - Results outside ±2,58σMAD = 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%
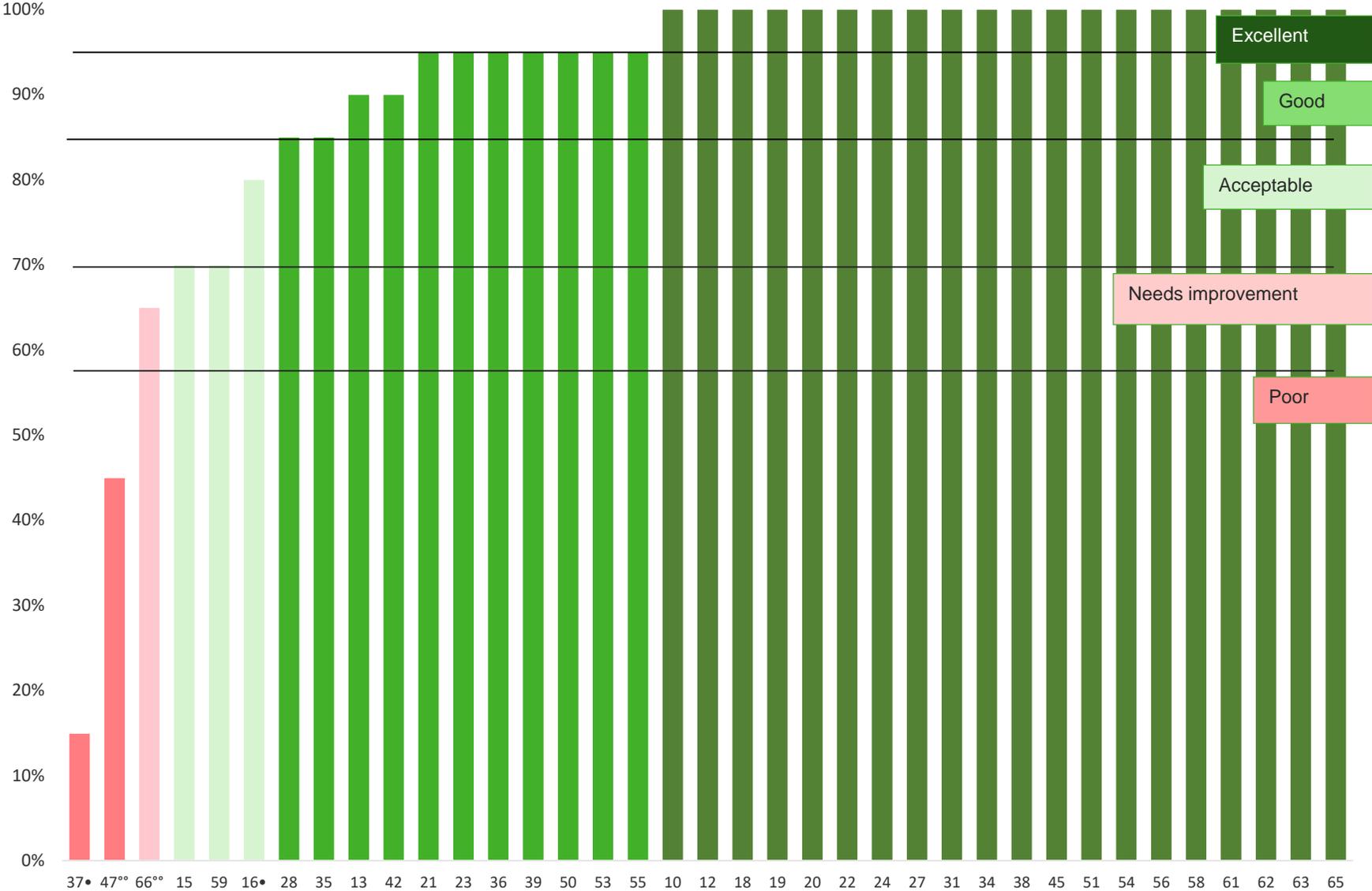
PERFORMANCE PT 21 (8 CAMPY+ SAMPLES)
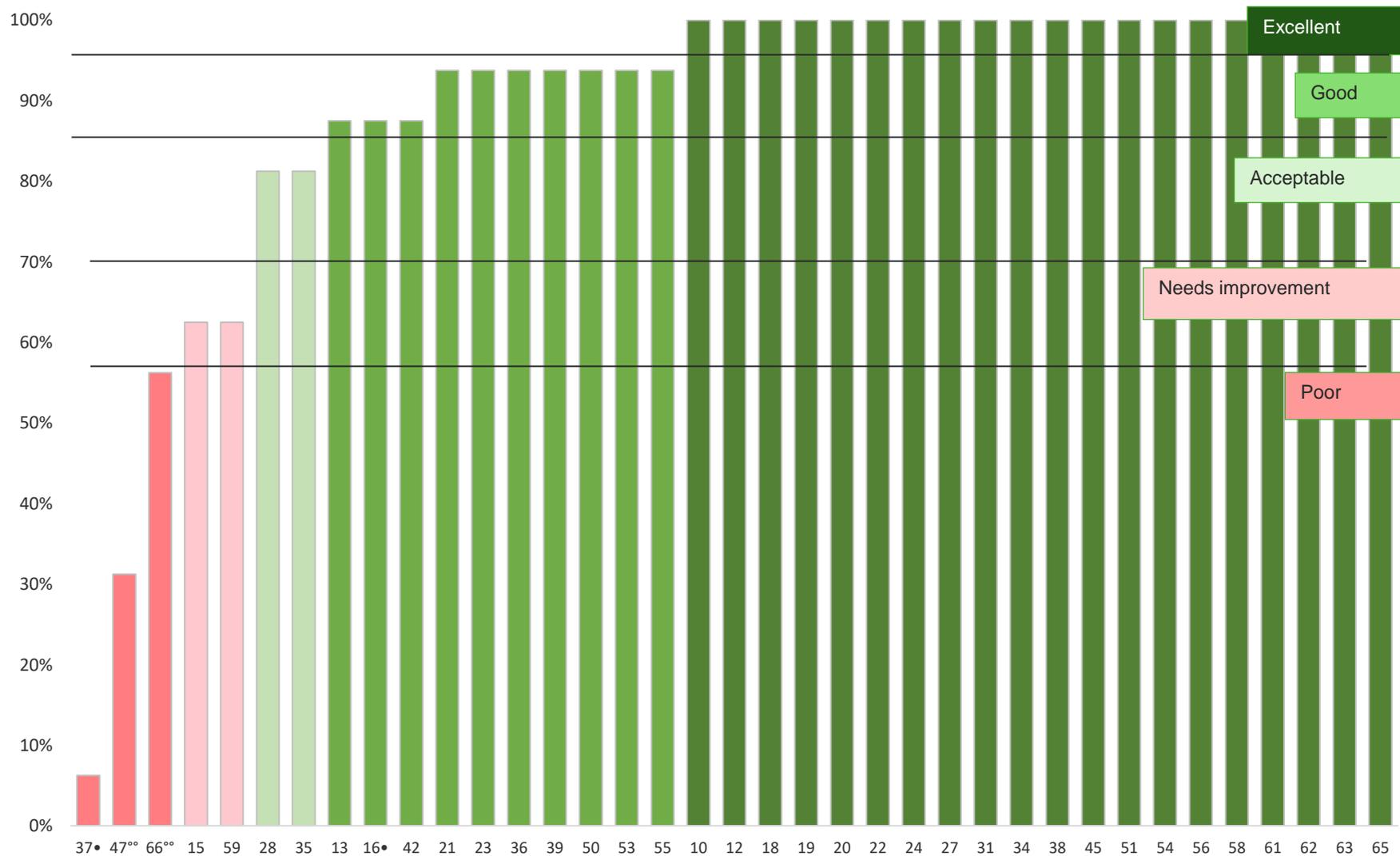
PT 21: PERFORMANCE IN RELATION TO START OF ANALYSIS

Day	No of NRLs	Performance				
		Excellent	Good	Acceptable	Needs improvement	Poor
6th of March	2	2				
7th of March	13	7	4	1		1
8th of March	2		2			
9th of March	2		2			
12th of March	8	5	2		1	
13th of March	3	2		1		
14th of March	4	2	1	1		
19th of March	1					1
21st of March	1	1				
26th of March	1	1				

PERFORMANCE IN ENUMERATION OVER TIME
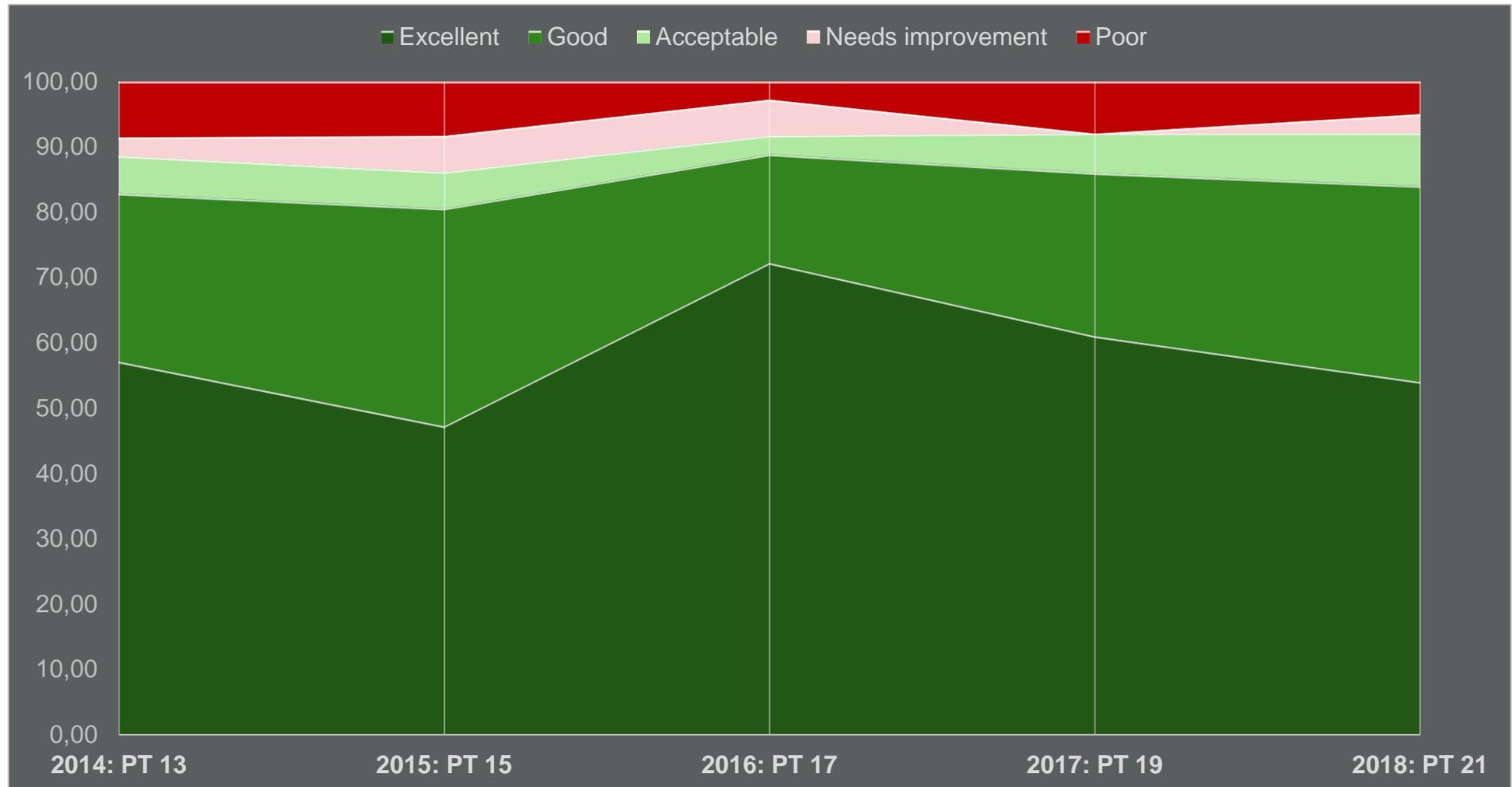
PT 21: SPECIES IDENTIFICATION (VOLUNTARY)

Content of sample (vial)	C. jejuni	C. coli	C. lari	Camp spp.	Other / No growth
1. Negative					33
2. *C. lari*			32	1	
3. *C. lari*			31	2	
4. E. coli		1			32
5. *C. coli* + E. coli		33			
6. *C. jejuni*	33				
7. *C. coli*	1	32			
8. *C. jejuni*	33				
9. *C. jejuni*	33				
10. *C. jejuni*	33				

PERFORMANCE PT 21:
SENSITIVITY IN DETECTION AND IDENTIFICATION OF *CAMPYLOBACTER* (VOLUNTARY)

DETECTION

SPECIES IDENTIFICATION

Good
6%

Acceptable
3%

Excellent
100%

Excellent
91%

SVA
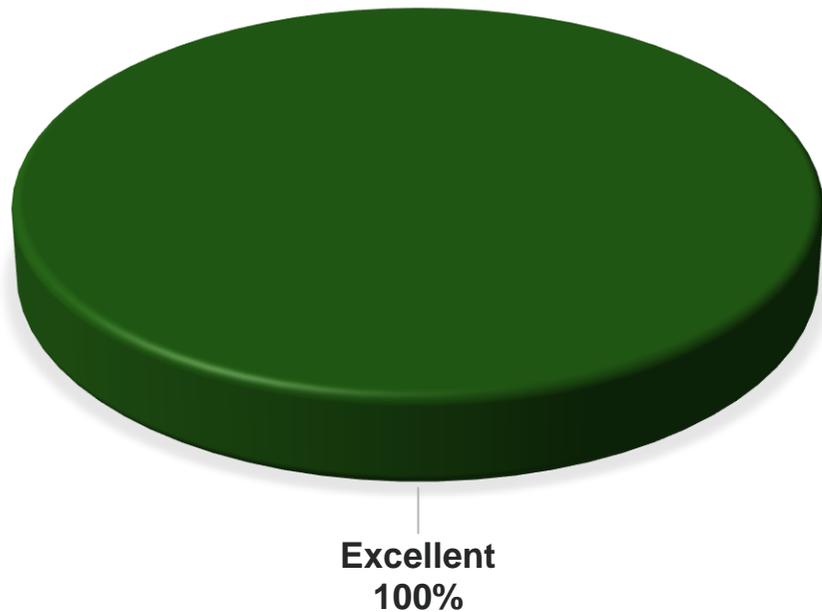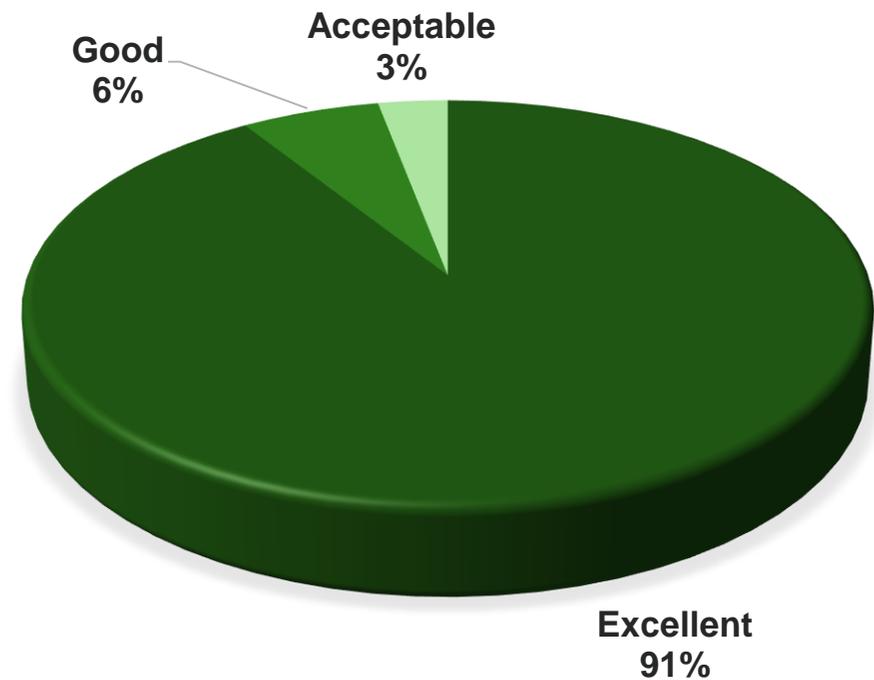
PERFORMANCE IN DETECTION (SE) OVER TIME
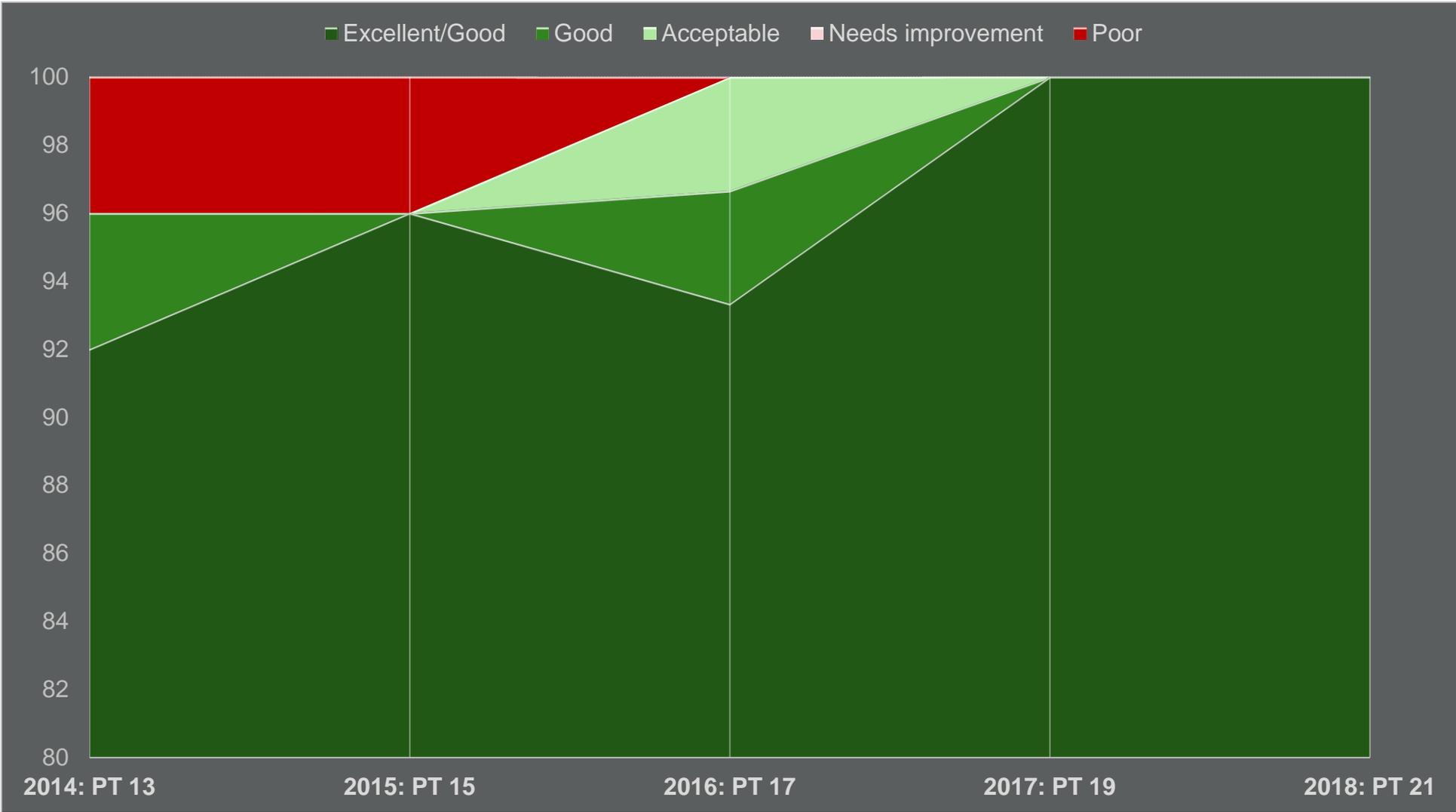
PERFORMANCE IN IDENTIFICATION (SE) OVER TIME
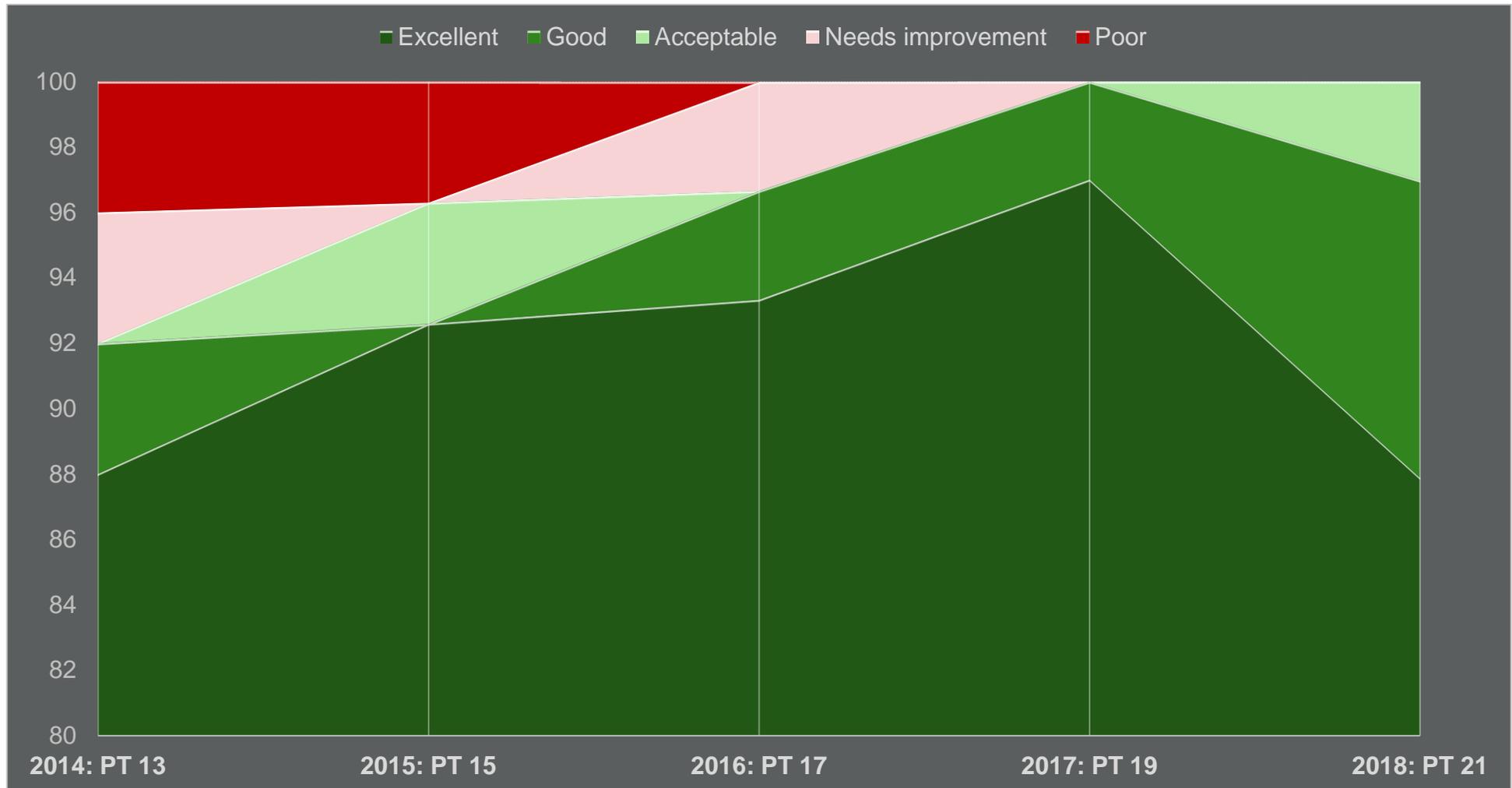
PT 22 – DETECTION AND SPECIES IDENTIFICATION OF *CAMPYLOBACTER*

PROFICIENCY TEST NO. 22

The objective was to assess the performance of the NRLs to detect and identify *Campylobacter* species in chicken faecal swab samples.

- Detection of *Campylobacter* spp. in chicken faecal swab samples

- Species identification of *Campylobacter*

- 18 core samples (mandatory) mimicking swabs taken from birds kept indoors

- 4 educational samples (voluntary and not included in the performance evaluation) mimicking swabs taken from birds kept outdoors

- Recommended method ISO 10272:2017, but other methods allowed

- No direction regarding which procedure (A, B or C) in the ISO method to use

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PT 22: CONTENTS AND PROCEDURE

- 22 E-swabs with chicken faecal material (with or without *Campylobacter*) in Cary Blair broth

- 22 vials with freeze-dried sample (with or without *Campylobacter*)

- Mix each vial with the content of the corresponding E-swab

- Follow the method(s) of choice for
    - detection
    - species identification

of *Campylobacter* spp.

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PT 22: CORE SAMPLES

Sample No.	Content in vial	Hippurate	Level	Content in E-swab
11	*Campylobacter coli*		High	
12	*Campylobacter coli*		Low	
13	*Campylobacter jejuni*	+	Low	*Escherichia coli*
14	Negative			
15	Negative			
16	Negative			
17	*Campylobacter jejuni*	+	High	*Escherichia coli*
18	Negative			*Escherichia coli*
19	*Campylobacter jejuni*	+	Low	
20	*Campylobacter jejuni*	+	High	*Candida*
21	*Campylobacter lari*		High	*Escherichia coli*
22	Negative			*Candida*
23	*Campylobacter jejuni*	+	High	
24	*Campylobacter coli*		High	*Escherichia coli*
25	*Campylobacter lari*		Low	*Candida*
26	Negative			*Escherichia coli*
27	*Campylobacter jejuni*	+	Low	*Escherichia coli*
28	*Campylobacter lari*		Low	

PT 22: EDUCATIONAL SAMPLES

Sample No.	Content in vial	Level	Content in E-swab
29	*Campylobacter upsaliensis*	High	
30	*Campylobacter lari*	High	
31	*Campylobacter coli*	Low	*Campylobacter jejuni* hipp+
32	*Campylobacter hyointestinalis*	High	
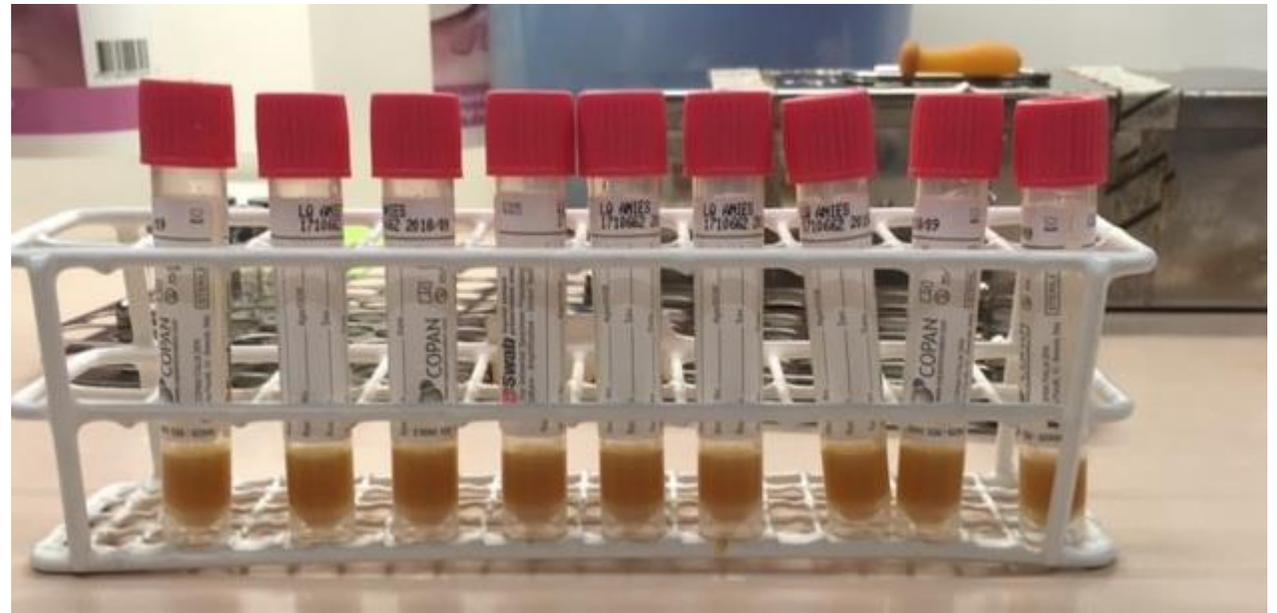
PT 22: QUALITY CONTROL
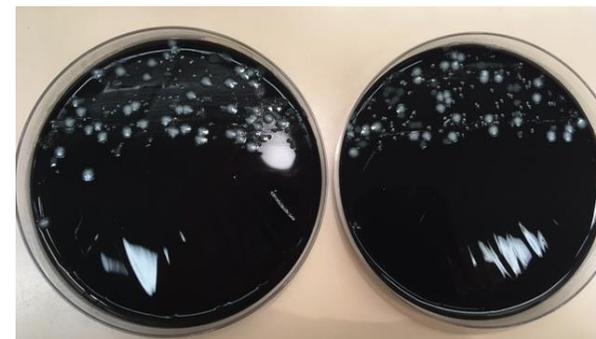
- Vials produced by EURL or the National Food Agency

- Tested for homogeneity and stability by the producer

- *Campylobacter* (*C. jejuni*) and non-*Campylobacter* (*E. coli*, *Candida* spp.) strains were tested for use as live cultures

- Pre-tests: vials together with matrix (E-swabs with or without added background flora) analysed according to ISO 10272-1:2017, procedure C (direct plating) and B (Preston)

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PT 22: PREPARATION OF THE TEST

Swab samples were prepared to resemble chicken cloacal swab samples

- E-swabs were emptied of their existing content

- Overnight cultures were prepared

- Caeca were cut and placed in a stomacher bag and mixed with Cary Blair transport medium

- A dilution of each overnight culture was mixed with the caecum suspension

- Each E-swab was filled with 1 ml of caecum suspension (with or without added bacteria)
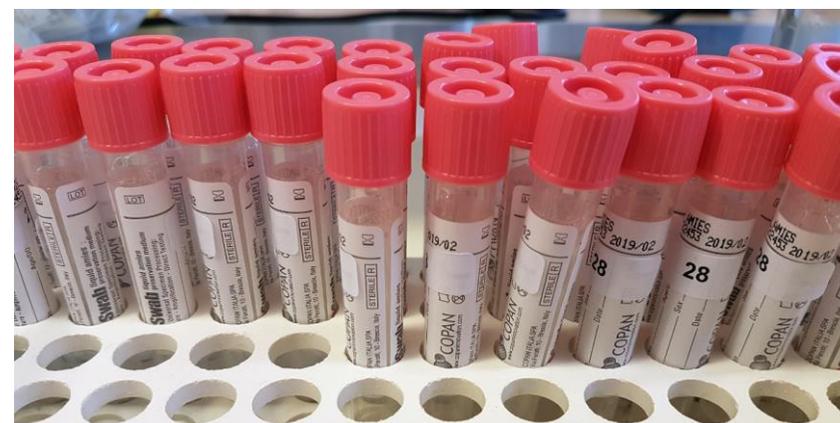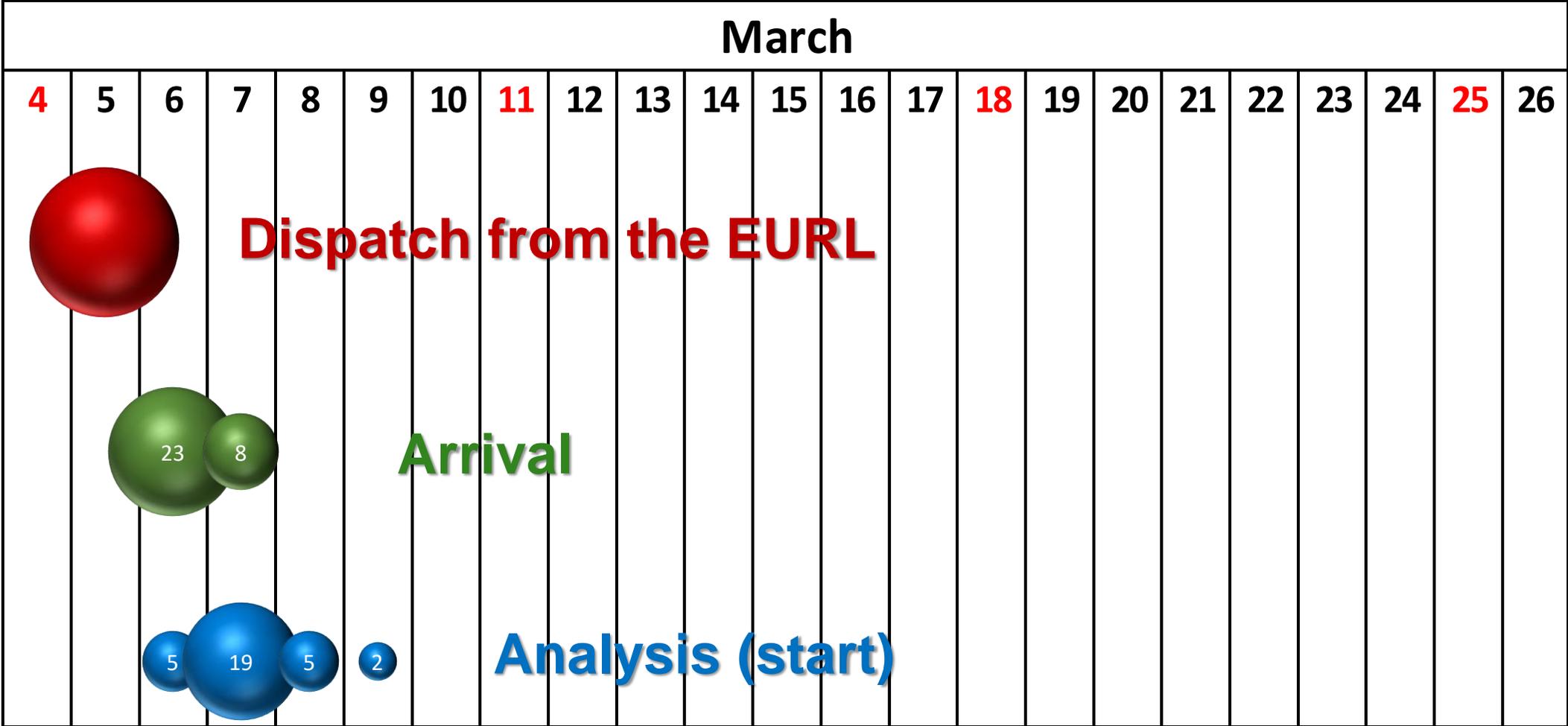
PT 22: METHODS

Reported method for detection	No. of NRLs
ISO 10272:2017	27
ISO 10272:2006	1
NMKL 119, 3rd ed. 2007	1
Other methods	2

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PT 22: PROCEDURES

Reported procedure(s) for detection	No. of NRLs		
Enrichment in Bolton broth (A)	10		
Enrichment in Preston broth (B)	9		
Direct plating (C)	23		
Enrichment in Exeter broth (D)	1		
Only direct plating	13		
Both direct plating and enrichment	10		
Only enrichment	8		
A	4	A+B	2
B	1	B+C	6
C	13	A+C	4
D	1		

PT 22: CORRECT REPORTED RESULTS PER SAMPLE IN DETECTION AND SPECIES IDENTIFICATION

PT 22: OVERALL SENSITIVITY IN DETECTION FOR (HIGH AND) LOW LEVEL SAMPLES

Samples	Se
All *Campylobacter*-postive samples, all labs	94.9 %
High level samples (11, 17, 20, 21, 23, 24), all labs	98.4 %
Low level samples (12, 13, 19, 25, 27, 28), all labs	91.4 %
Low level samples, labs using only direct plating (13)	88.5 %
Low level samples, labs using only enrichment (8)	89.6 %
Low level samples, labs using both principles (10)	96.7 %

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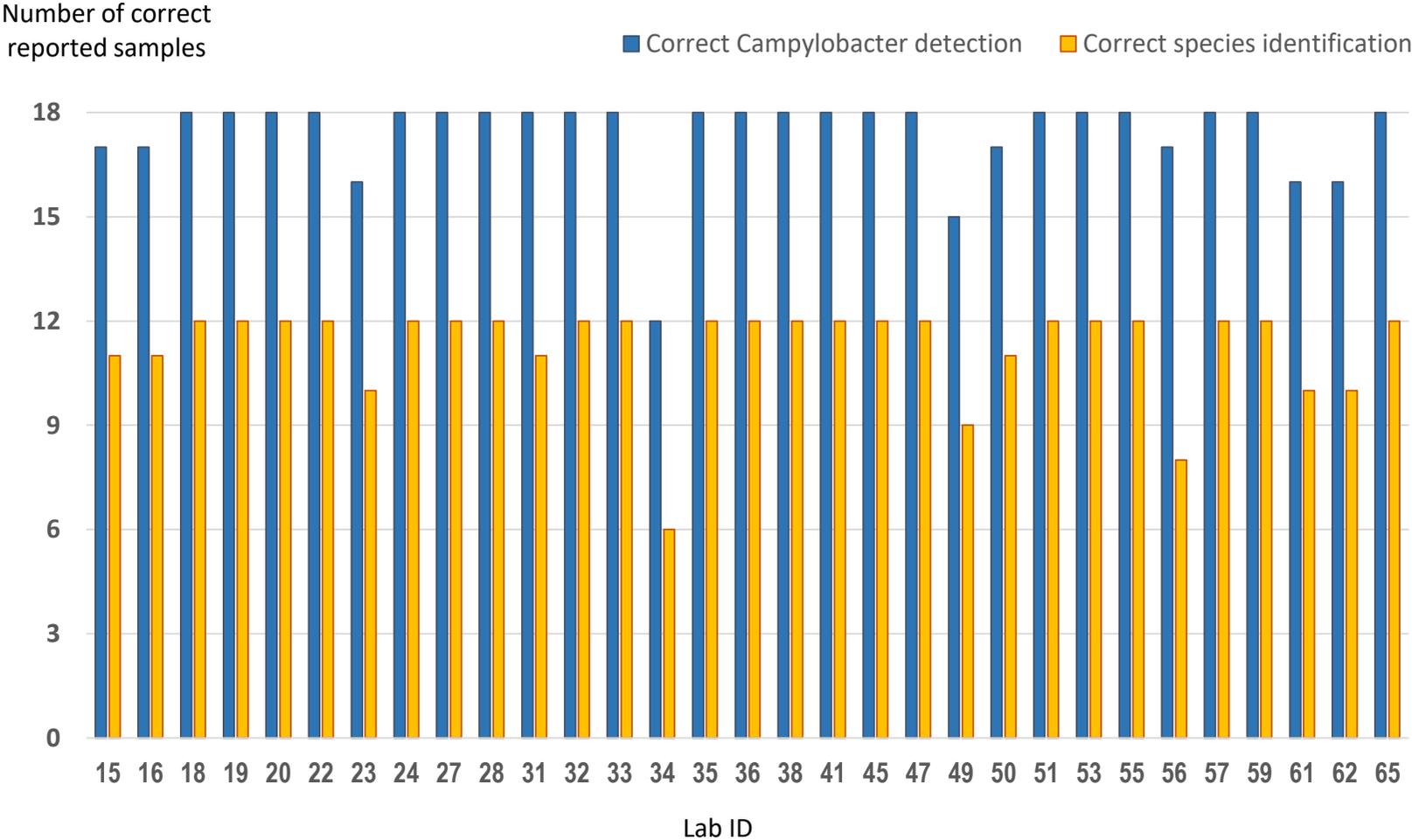
PT 22: PERFORMANCE – SE AND SP IN DETECTION OF *CAMPYLOBACTER*

DETECTION *CAMPYLOBACTER*
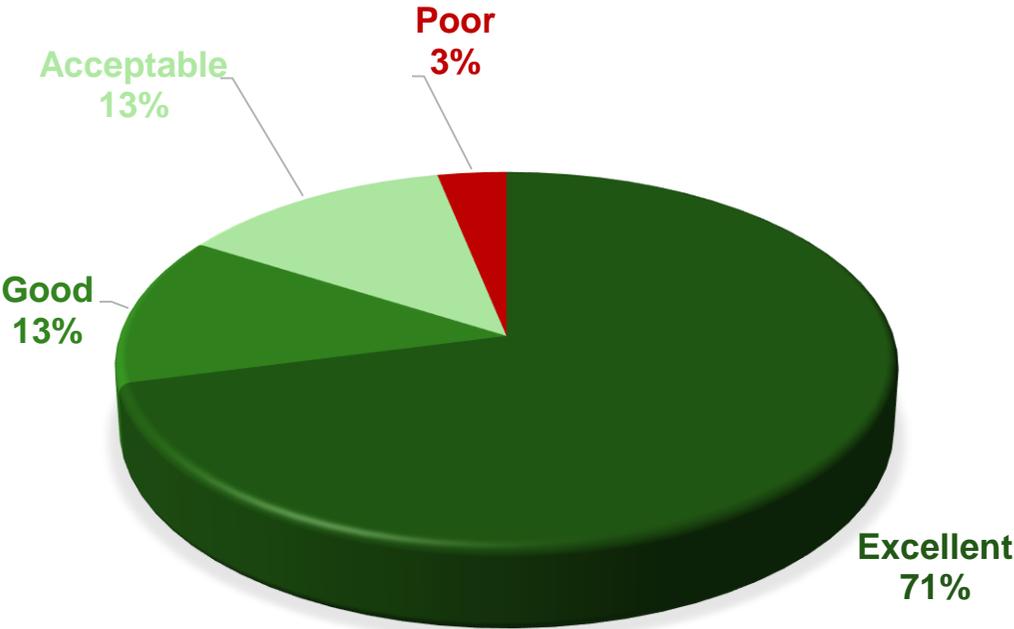
DETECTION NON-*CAMPYLOBACTER*
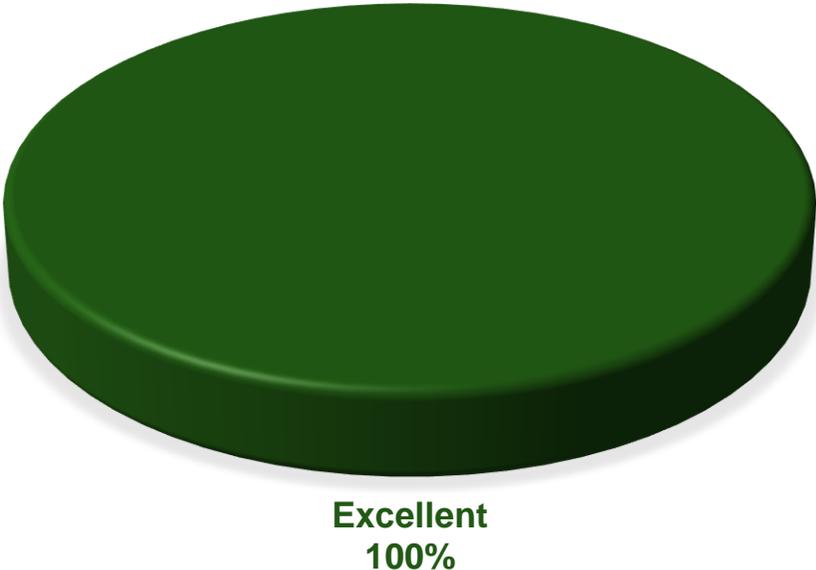
PT 22: ACCURACY IN DETECTING POSITIVE AND NEGATIVE *CAMPYLOBACTER* SAMPLES

Poor
3%

Acceptable
13%

Good
13%

Excellent
71%

SVA
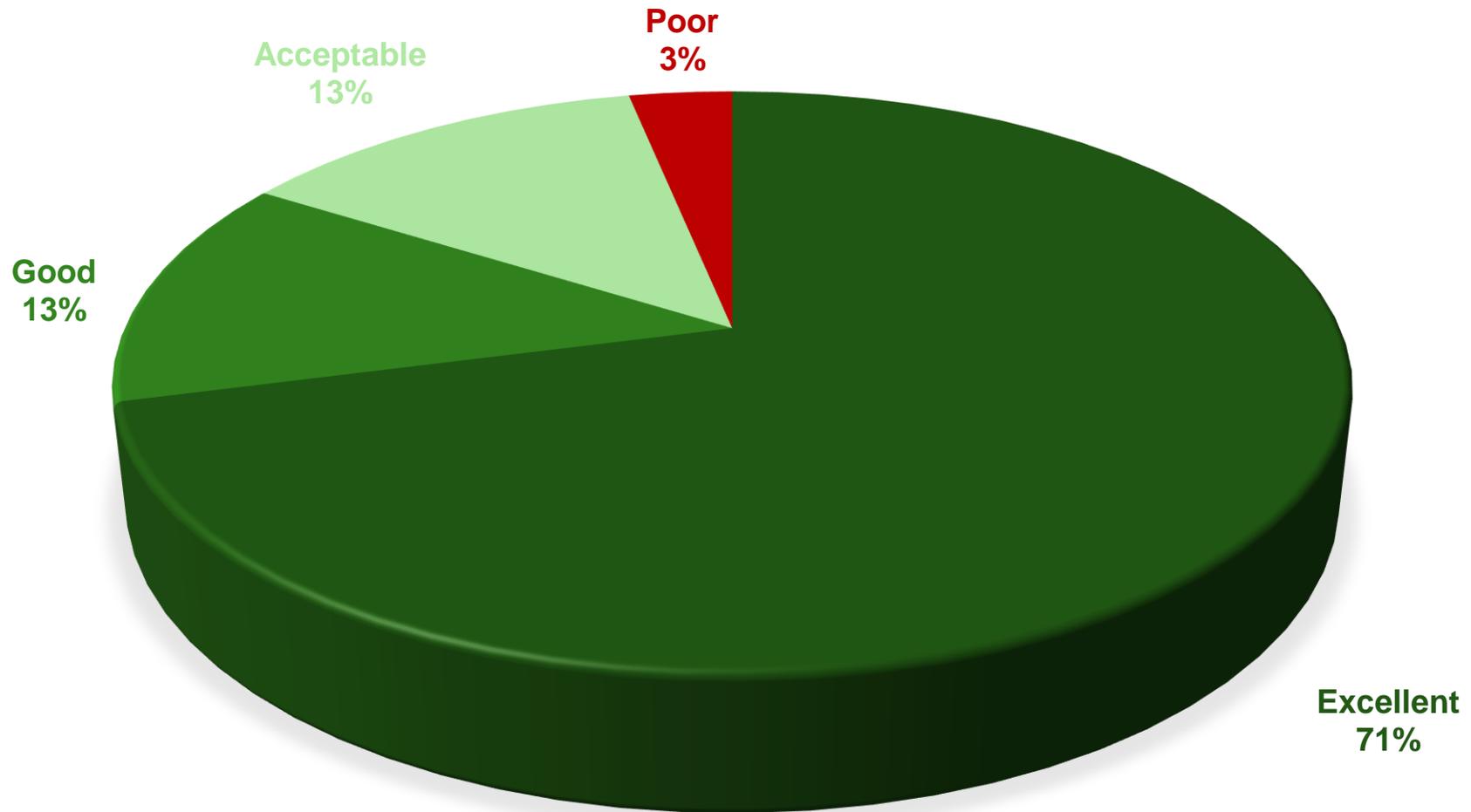
ACCURACY – COMPARISON WITH PREVIOUS TESTS
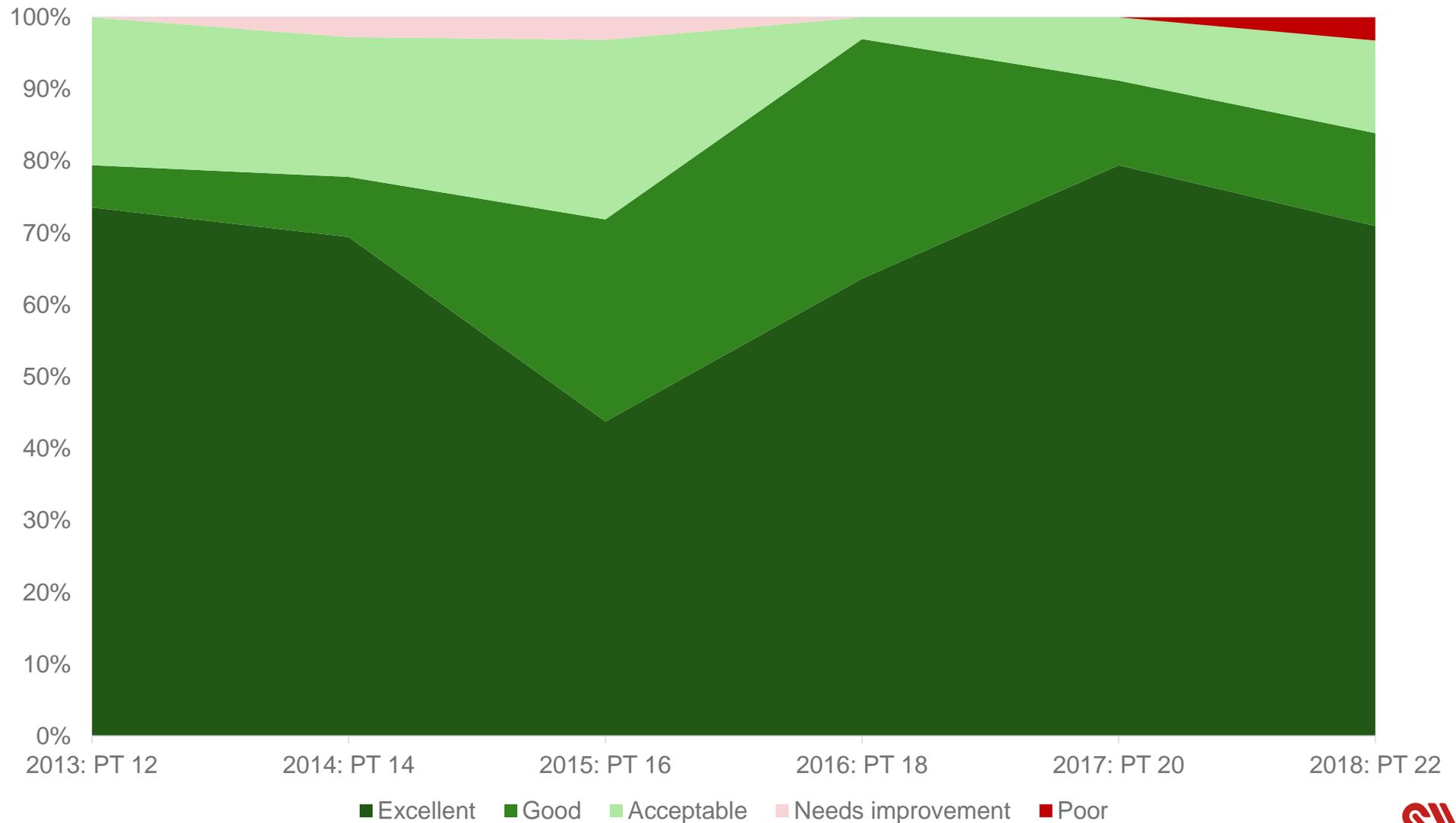
PT 22: REPORTED SPECIES IDENTIFICATION

Sample No.	Bacterial species	Hippurate hydrolysis	C. jejuni	C. coli	C. lari	Campylobacter spp. but unable to identify species	Growth of other, not Campylobacter	No growth at all
11	*Campylobacter coli*			31				
12	*Campylobacter coli*			30			1	
13	*Campylobacter jejuni*	+	29				2	
14	Negative						11	20
15	Negative						10	21
16	Negative						13	18
17	*Campylobacter jejuni*	+	30				1	
18	*Escherichia coli*						29	2
19	*Campylobacter jejuni*	+	31					
20	*Campylobacter jejuni*	+	31					
21	*Campylobacter lari*				29	1	1	
22	*Candida* spp.						22	9
23	*Campylobacter jejuni*	+	31					
24	*Campylobacter coli*			30			1	
25	*Campylobacter lari*				23	1	6	1
26	*Escherichia coli*						27	4
27	*Campylobacter jejuni*	+	28		1		2	
28	*Campylobacter lari*				26	1	2	2

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PT 22: PERFORMANCE – SENSITIVITY SPECIES IDENTIFICATION
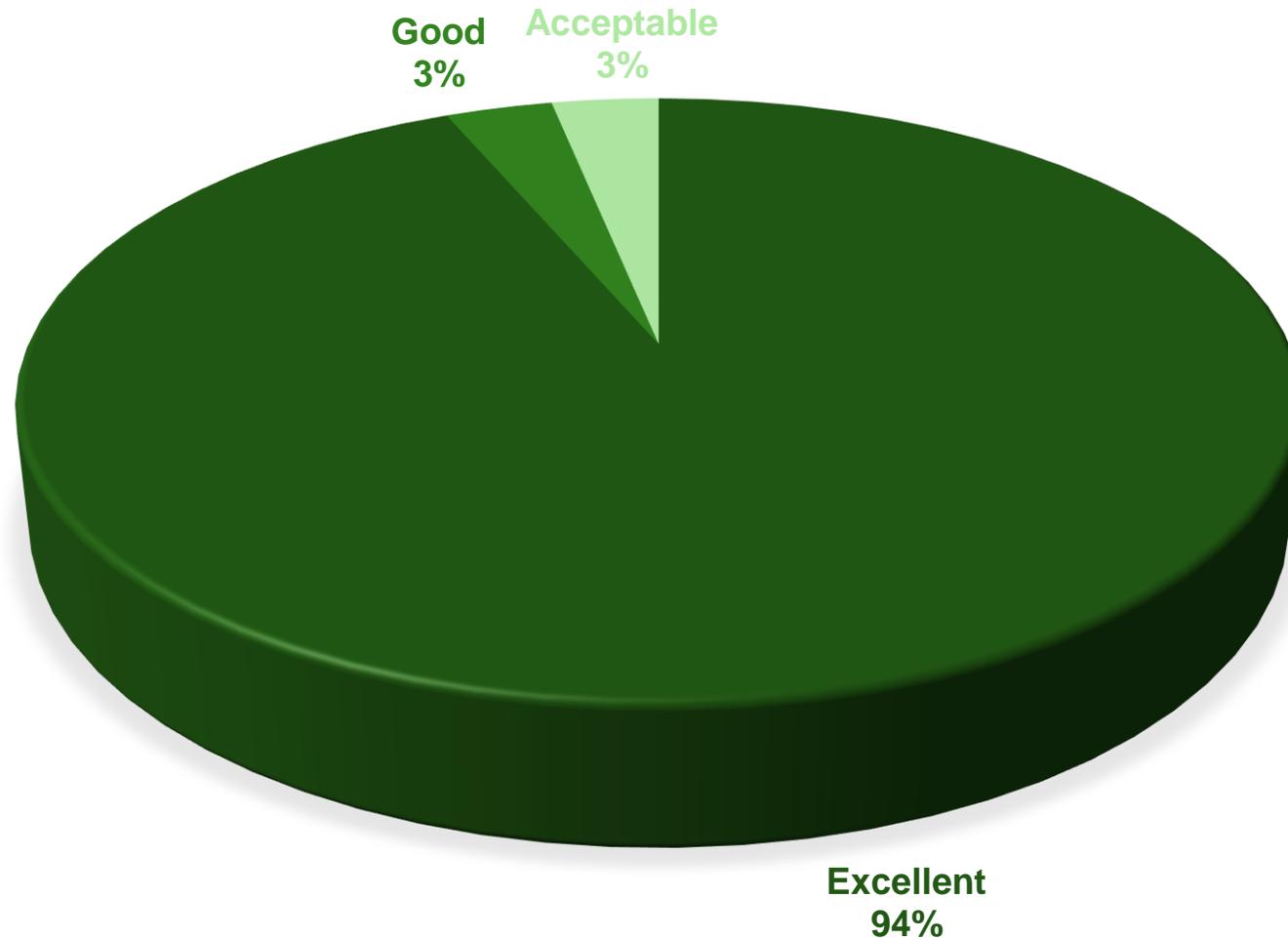
IDENTIFICATION – COMPARISON WITH PREVIOUS TESTS

■ Excellent	■ Good	■ Acceptable	■ Needs improvement	■ Poor	
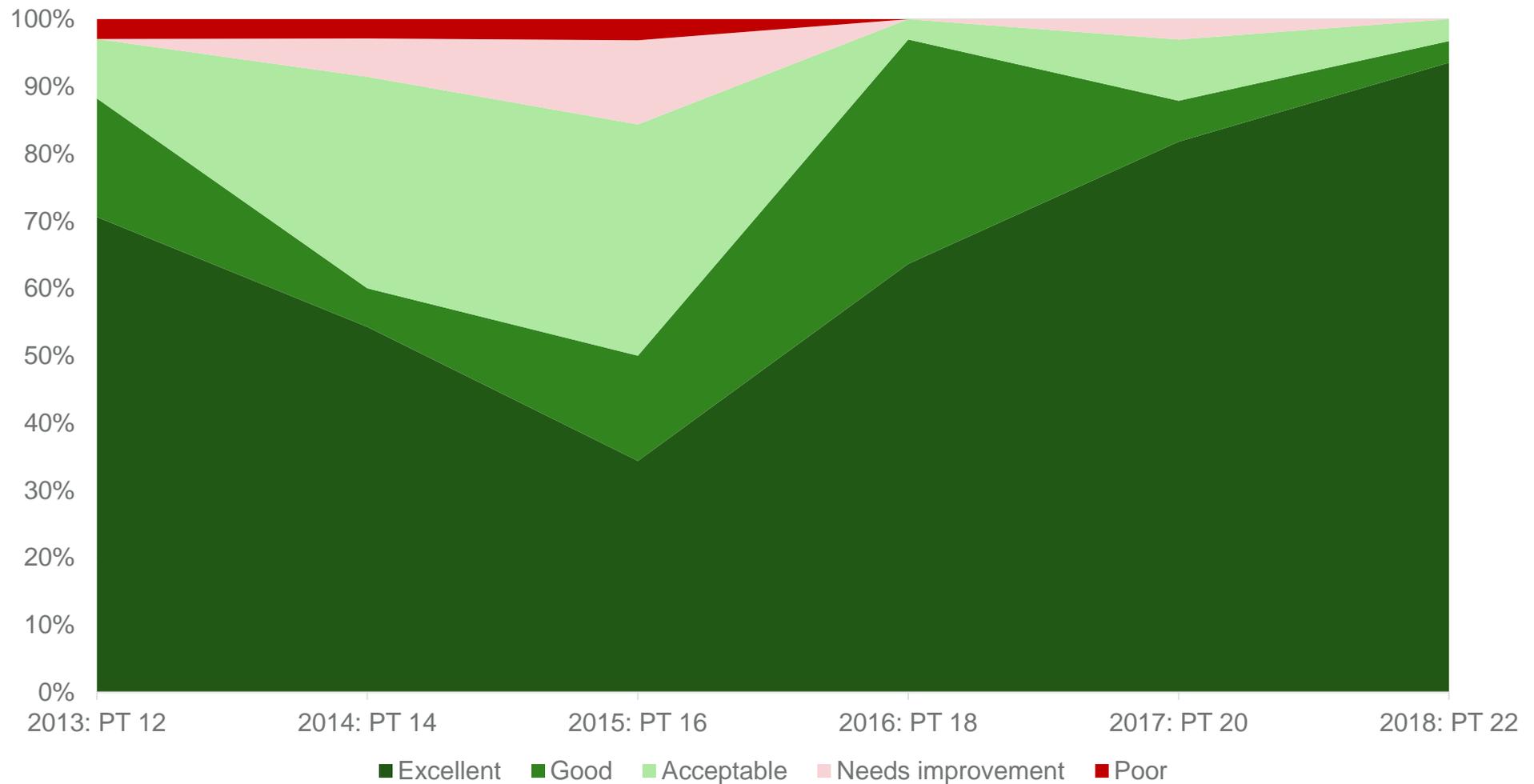
PT 22: NUMBER OF CORRECT SPECIES IDENTIFICATIONS IN SAMPLES WITH *CAMPYLOBACTER* BY DIFFERENT METHODS

Method for species identification	Correct Sp id in all samples analysed	Total
Biochemical tests only	4	4
PCR assays only	**2**	3
MALDI-TOF only	**9**	10
PCR and biochemical tests	8	8
PCR and MALDI-TOF	3	3
MALDI-TOF and biochemical tests	2	2
Biochemical tests, PCR and MALDI-TOF	1	1

PT 22: EDUCATIONAL SAMPLES

Sample No.	Bacterial species	*C. jejuni*	*C. coli*	Both *C. jejuni* and *C. coli*	*C. lari*	*C. upsaliensis*	*C. helveticus*	*C. hyointestinalis*	*C. fetus*	*Campylobacter* spp. but unable to identify species	No *Campylobacter* detected
25	*Campylobacter upsaliensis*	1				**9**	3			2	16
26	*Campylobacter lari*	1			**29**						1
27	*Campylobacter coli* *Campylobacter jejuni*	17	4	**8**	1						1
28	*Campylobacter hyointestinalis*	1			1			**17**		3	8

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PT 22: OVERALL SENSITIVITY AND PERFORMANCE RATE FOR EDUCATIONAL SAMPLES

Sample No.	Campylobacter species	Sensitivity in detection	Sensitivity in species id	Combined performance rate
29	*C. upsaliensis*	48.4%	60.0%	38.7%
30	*C. lari*	96.8%	96.7%	95.2%
31	*C. coli* + *C. jejuni*	96.8%	61.7%	78.2%
32	*C. hyointestinalis*	74.2%	73.9%	64.5%
All		79.0%	75.0%	69.2%

COMMENTS AND QUESTIONS

- Which procedure (A, B, C) is adequate?

- How should the enrichment for the voluntary detection in PT 21 be prepared after preparing the initial suspension according to the instructions?

- Reporting in Questback

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