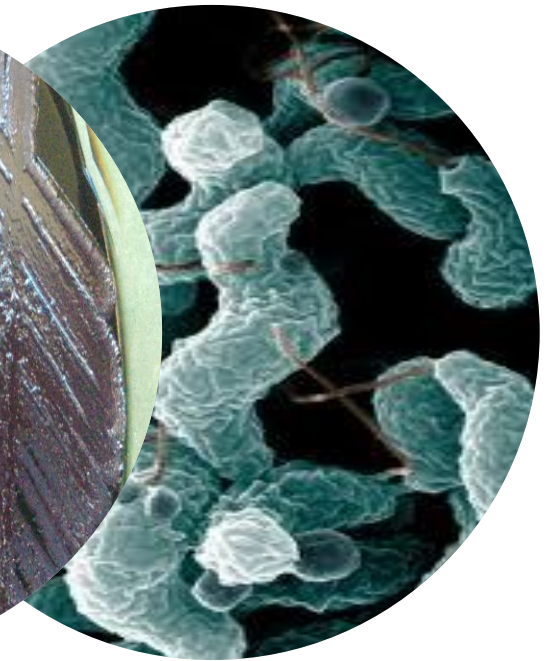
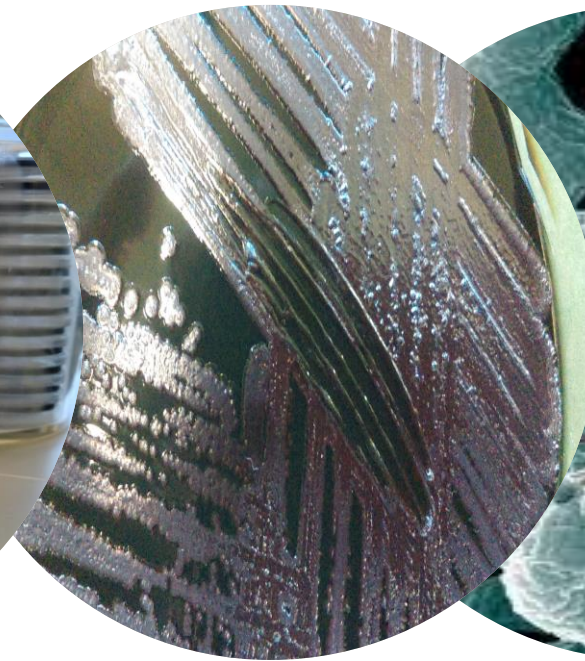


organizing PT's, lessons learned@WBVR (NL)

EU-RL workshop 2021

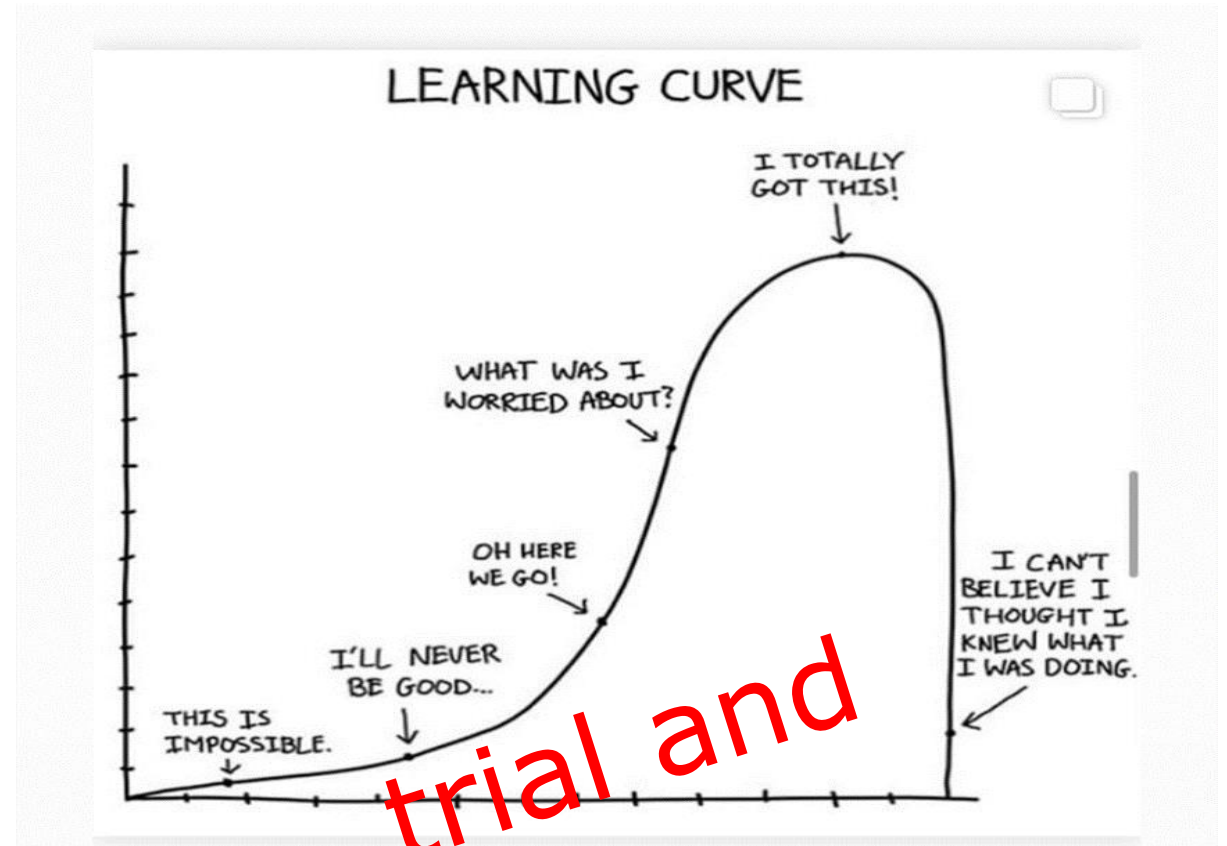
Sept 29th, Miriam Koene



A short history of PT's at WBVR, a steep learning curve

- small NRL team

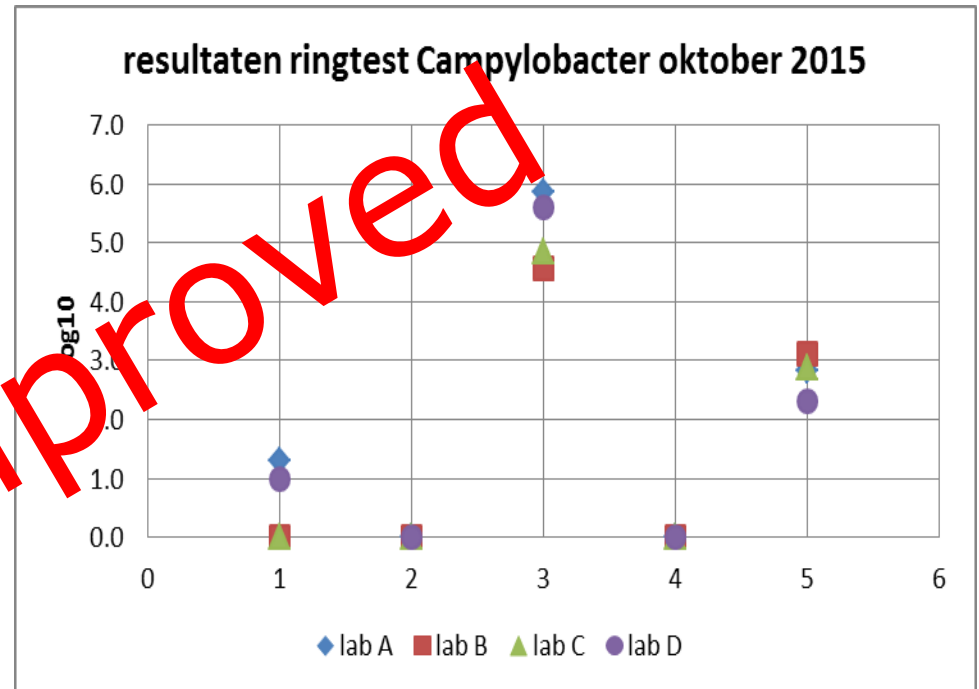
- Conny van Solt and Miriam Koene (from 2014)
- Ria van der Hulst and Frans Puturilan (2007-2013)



trial and error

How did we start?

- 2015 request from 2 external laboratories for a PT on **enumeration** for accreditation purposes
- Organisation of a 'mini-PT'
 - Dilutions of WBVR *C. jejuni* reference material in cryovials
 - 5 samples
 - Levels: blank, log2, log3, log5, log7
- Reporting: descriptive, no performance criteria used



WBVR participated 'twice' (at/after dispatch of samples)

Lesson learned:

decay of Campy to be taken into account

First PT on detection and identification (1)

- 7 participating labs
- 10 samples; *C. jejuni* (4), *C. coli* (2), negative/blanks (4), including *Arcobacter*
- artificially contaminated caecal contents and BHI (fresh culture) plus naturally contaminated caecal contents from a field study
- swabs
- courier, cooled



File for submission of results

Excellfile (with dropdown menu)

Samples tested by organising lab at and after dispatch

A	B	C	D	E	F	G	
Uitslagen rapport							
Ringonderzoek detectie van <i>Campylobacter</i> maart 2017							
Naam laboratorium: WBVR							
Naam contactpersoon: Albert de Boer							
E-mail contactpersoon:							
Opmerking							
Datum en tijd aankomst van de monsters:				21/3/2017			
Conditie van de verpakking:				ok			
Zijn de koelelementen nog koud:				ja			
Methode voor Microaeroob milieu:				Commercieel gas pak			
Ophopingsmedium 1				Ophopingsmedium 2		Selectieve plaat 1	Selectieve plaat 2
Gebruikte media: preston						CCDA	
Controle stammen (Species, stamnummer):				Campylobacter jejuni			
Methode voor conformatie verdachte kolonies							
Opmerking							
Microscopisch morfologie en motiliteit:				Ja			
Microaerobe groei bij 25°C:				Ja			
Oxidase test:				Ja			
Maldi-TOF MS:				Nee			
PCR (naam): Biorad IQ Check				ja			
Anders:							
Datum en tijd van start onderzoek: 21/3/2017							
Analyseresultaten							
Monster nr.	Groei	Verdacht	Bevestigd	Campylobacter PCR	Campylobacter traditioneel	Opmerkingen	
C1	nee	Nee	Nee	Negatief	Negatief		
C2	ja	nee	Nee	Negatief	Negatief		
C3	ja	Nee	Nee	Positief	Negatief		
C4	ja	ja	ja	Positief	Positief		
C5	ja	ja	ja	Positief	Positief		
C6	nee	nee	Nee	Negatief	Negatief		
C7	ja	nee	Nee	Negatief	Negatief		
C8	ja	ja	ja	Positief	Positief		
C9	ja	ja	ja	Positief	Positief		
C10	nee	nee	Nee	Negatief	Negatief		

First PT on detection and identification (3)

■ Lessons learned:

- use of naturally contaminated caecal samples is 'high risk' material in terms of PTs
- use of fresh culture material for spiking also risky
- safer to use high levels of contamination
- matrix availability can be challenging

matrix	Species	level	Score	LAB A enrichment	LAB B enrichment	LAB C direct	LAB D direct	LAB E direct	LAB F enrichment	LAB G enrichment	LAB G PCR
BHI	C. jejuni	low	Positive (P)	P	P	P	P	P	P	N	N
caecal contents	-	-	Negative (N)	N	N	N	N	N	N	N	N
BHI	C. jejuni	high	Positive (P)	P	P	P	P	P	P	N	P
caecal contents	C. jejuni	naturally contaminated	Positive (P)	P	P	N	N	N	P	P	P
caecal contents	Arcobacter	-	Negative (N)	N	N	N	N	N	N	P	P
BHI	C. coli	low	Positive (P)	N	P	N	N	N	P	N	N
caecal contents	E. coli	-	Negative (N)	N	N	N	N	N	N	N	N
caecal contents	C. jejuni	naturally contaminated	Positive (P)	P	P	N	N	N	P	P	P
BHI	C. coli	high	Positive (P)	N	P	P	P	P	P	P	P
BHI	-	-	Negative (N)	N	N	N	N	N	N	N	N

To be improved

Next attempt enumeration

■ 8 laboratories

- Freeze dried Reference material RM-micro
- 5 samples; *C. jejuni* (3), *C. coli* (1), blank (1)
- Chicken skin (Campy negative)
- courier, cooled, datalogger



Tabel 1. Samenstelling ringtestpanel

ID	omschrijving	cfu/ampul	cfu/gram huid
148	Reincultuur <i>C. jejuni</i>	1.5 log ₁₀	0.5 log ₁₀
258	Reincultuur <i>C. jejuni</i>	2.3 log ₁₀	1.3 log ₁₀
288	Reincultuur <i>C. jejuni</i>	6.3 log ₁₀	5.3 log ₁₀
287	Reincultuur <i>C. coli</i>	5.6 log ₁₀	4.6 log ₁₀
272	Blanco	negatief	0



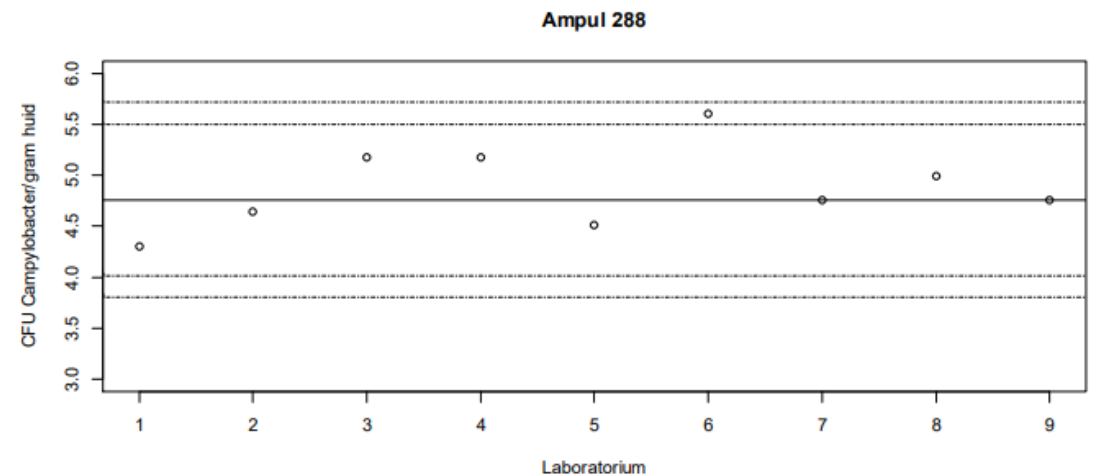
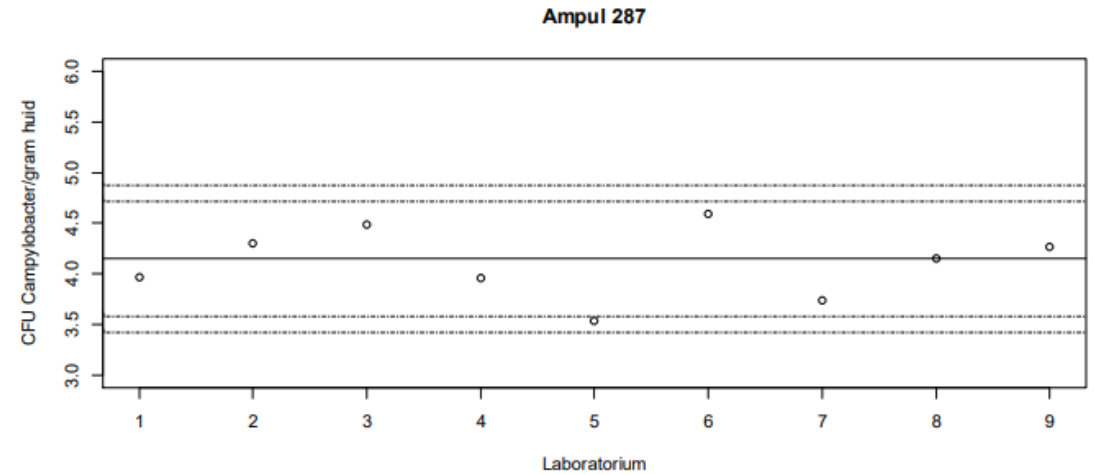
■ Results

sample	Laboratory							
	A	B	C	D	E	F	G	H
148	<10	<10	<10	20	90	<10	<10	10
258	20	<40	<10	<10	60	<10	10	<10
272	<10	<10	<10	<10	80	<10	<10	<10
287	2.0E+04	3.9E+04	5.0E+03	3.1E+04	9.2E+03	3.5E+03	1.85 * 10 ⁴	1.4 * 10 ⁴
288	4.4E+04	4.0E+05	5.0E+04	1.5E+05	1.5E+05	3.2E+04	5.7 * 10 ⁴	9.7 * 10 ⁴

Next attempt enumeration

Lessons learned

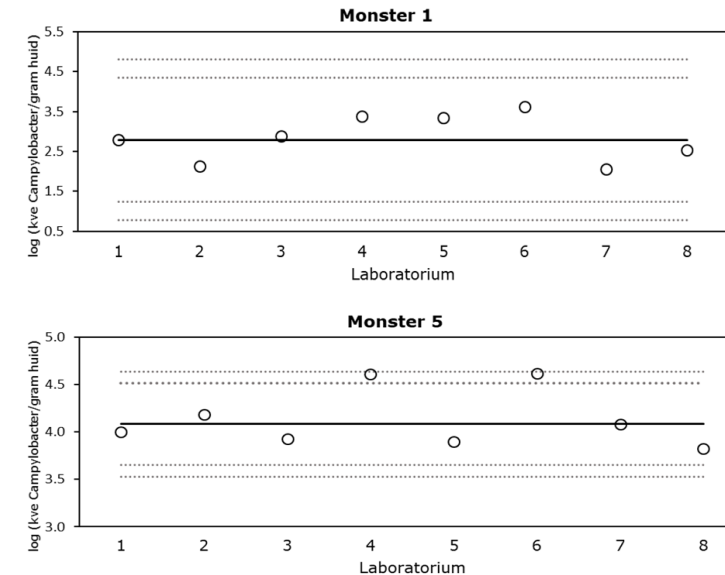
- dilution factor in matrix has to be taken into account
- commercial reference material is too expensive
- MAD scores work well
- Performance criteria implemented



Figuren 1 en 2. Overzicht van de resultaten van ampullen 287 en 288 voor de negen deelnemende laboratoria. De doorgetrokken lijn is de mediaan, de gestippelde lijnen zijn de mediaan $\pm 2 \delta_{MAD}$ en de mediaan $\pm 2.58 \delta_{MAD}$ grenzen.

Analysis and reporting

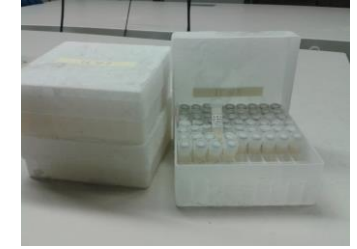
- Labs are free to use their own (accredited) method
- True value of *Campylobacter* concentration unknown: 'consensus value', based on median concentration as reported by all laboratories
- Median Absolute Deviation (MAD)
 - Result within median $\pm 2 \delta\text{MAD}$: 2 points
 - Result within $\pm 2 \delta\text{MAD}$ en $\pm 2.58 \delta\text{MAD}$: 1 point
 - Results outside this range: 0 points
- Performance criterium $\geq 70\%*$



Tabel 3. Median Absolute Deviation (MAD) scores per laboratorium.

Monster	1	2	3	4	5	6	7	8	9	10	Totaal (max)	Percentage
Lab 1	2	2	2	2	2	2	2	2	2	0	18 (20)	90
Lab 2	2	2	2	1	2	2	2	2	2	2	19 (20)	95
Lab 3	2	2	2	2	2	2	2	2	2	2	20 (20)	100
Lab 4	2	2	2	2	1	2	2	2	2	2	19 (20)	95
Lab 5	2	2			2	2	2	2	2	2	16 (16)	100
Lab 6	2	2	2	2	1	2	2	2	2	2	19 (20)	95
Lab 7	2	2	2	2	2	2	2	2	2	2	20 (20)	100
Lab 8	2	2	2	2	2	2	2	2	2	2	20 (20)	100

In-house produced reference material



- Improvements by implementing BfR procedure for cryovials

Stability testing: improved storage of lower levels of *Campylobacter*

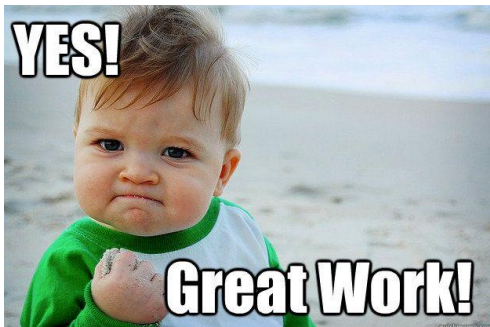
- Broader set of cryovials (strains and levels)
- Freeze drying: work to be continued



Further PT's

Detection (plus identification) and enumeration

- Cryovials WBVR/BfR
- Chicken skin (Campy negative)
- Format for analysis and reporting



Tabel 3. Scores per laboratorium

Monster	1	2	3	4	5	6	7	8	9	10	Totaal	Percentage
Lab 1	2	2	2	2	2	2	2	2	2	2	20	100
Lab 2	2	1	2	2	2	1	2	2	2	2	18	90
Lab 3	2	2	2	2	2	2	2	2	2	2	20	100
Lab 4	2	2	2	2	2	1	2	2	2	2	19	95
Lab 5	2	0	2	2	2	2	2	2	2	2	18	90
Lab 6	2	2	2	2	2	2	2	2	2	2	20	100
Lab 7	2	0	0	2	0	0	0 ^a	0	2	2	8	40
Lab 8	2	2	2	2	0 ^a	2	2	2	2	2	18	90
Lab 9	2	2	2	2	2	2	2	2	2	2	20	100

^a geen uitslag

Tabel 1. Samenstelling ringtestpanel

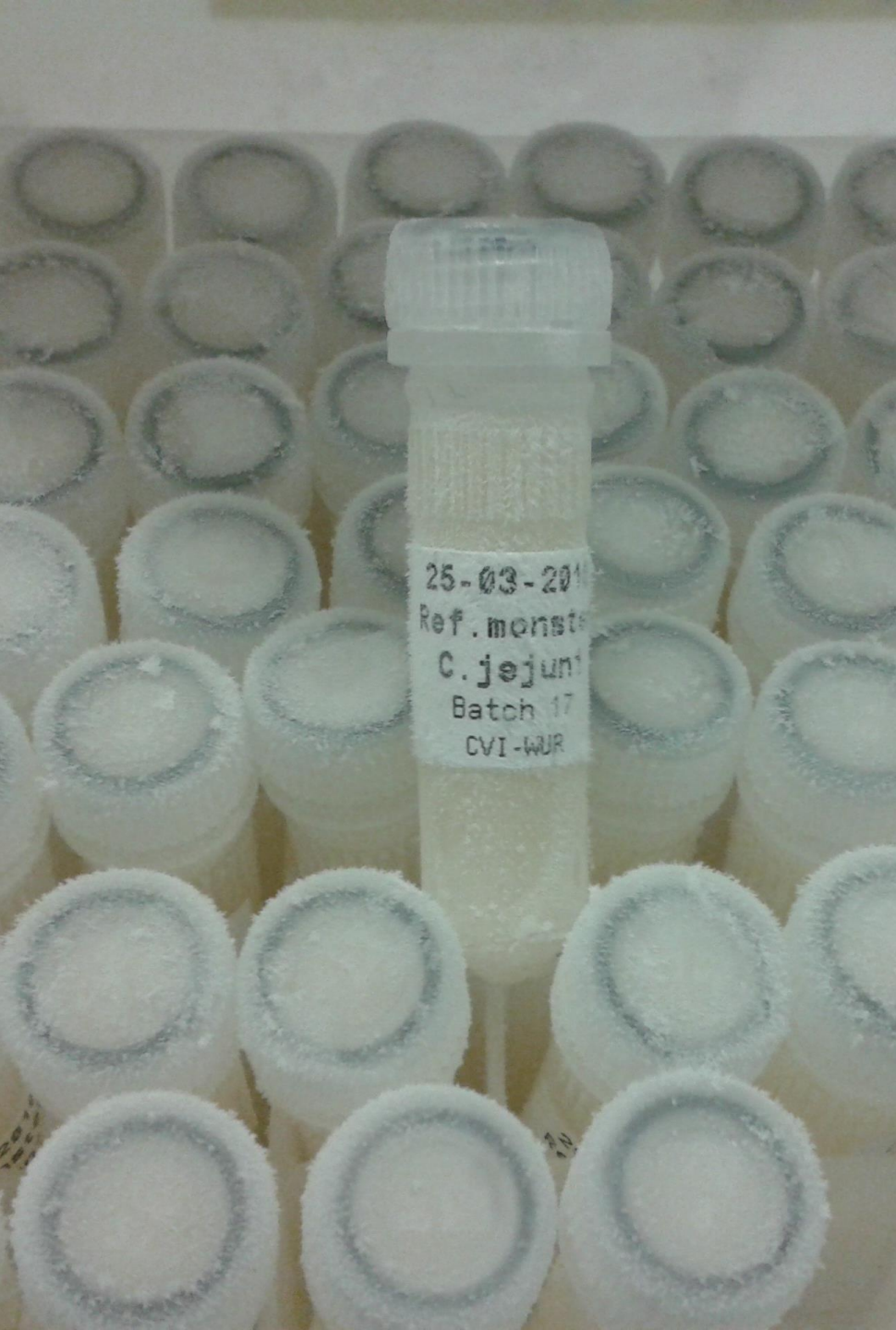
ID	omschrijving	niveau	Gemiddelde KVE/gram huid ¹
1	Reincultuur <i>C. jejuni</i>	laag	2,0 log ₁₀ ± 0,4
2	Reincultuur <i>C. jejuni</i>	hoog	5,1 log ₁₀ ± 0,5
3	Blanco	negatief	0
4	Reincultuur <i>C. jejuni</i>	middelhoog	3,0 log ₁₀ ± 0,4
5	Reincultuur <i>C. jejuni</i>	hoog	5,0 log ₁₀ ± 0,6
6	Reincultuur <i>C. coli</i>	middelhoog	2,8 log ₁₀ ± 1,2
7	Reincultuur <i>C. jejuni</i>	middelhoog	2,8 log ₁₀ ± 0,4
8	Blanco	negatief	0
9	Reincultuur <i>C. jejuni</i>	hoog	3,7 log ₁₀ ± 0,6
10	Reincultuur <i>C. coli</i>	hoog	4,1 log ₁₀ ± 0,4

¹ Gemiddelde waarde op basis van de resultaten van deze ringtest

Tabel 3. De prestaties (gevoeligheid en nauwkeurigheid) bij het detecteren van Campylobacter en niet-Campylobacter spp. en de prestatie (gevoeligheid) bij identificatie van Campylobacter spp. van de 6 deelnemers aan rondzendoefening 2019-1.

Lab id	Gevoeligheid in detectie*	Nauwkeurigheid in detectie	Gevoeligheid bij species identificatie
1	100%	100%	100%
2	86%	90%	100%
3	100%	100%	-
4	100%	100%	-
5	83%	90%	100%
6	86%	90%	-

* Deelnemers 5 en 6 hebben beide één positief monster als negatief gescand, deelnemer 5 had zes positieve monsters ontvangen, deelnemer 6 had zeven positieve monsters ontvangen. Dat geeft voor deelnemer 5 een gevoeligheid in detectie van 83% en voor deelnemer 6 een gevoeligheid van 86%.



Summarizing

- availability relevant matrices can be challenging
- use of naturally contaminated samples or fresh culture material for spiking is 'risky' (cannot be extensively tested prior to shipment)
- high levels of contamination are more safe to use
- EU-RL PT's have been a source of inspiration
- collaboration with other labs can be very helpful
- In-house production of reference material is possible

Questions, tips, other experiences?

