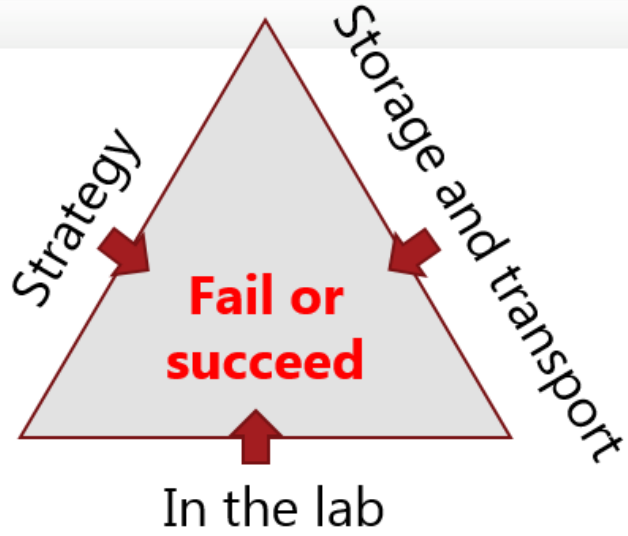
PRRSV Diagnostics – find the needle in the haystack!

Lars Erik Larsen
Professor UCPH

KØBENHAVNS UNIVERSITET

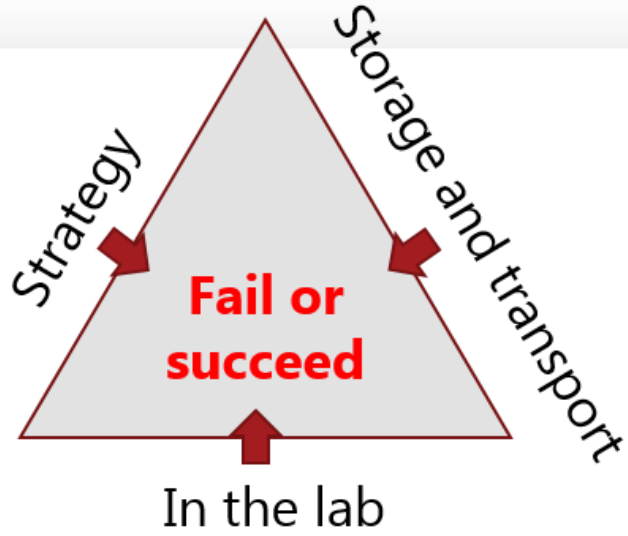
PRRSV detection – the diagnostic triangle!



- **Prior to sampling – decide strategy**
- **In the herd and to the laboratory**
- **In the laboratory**

Strategy
What is the goal and budget?
Consequence of false negatives
No of samples
Type of samples
Strategy (random, targeted,..)
Interval between samplings
Number of sample times

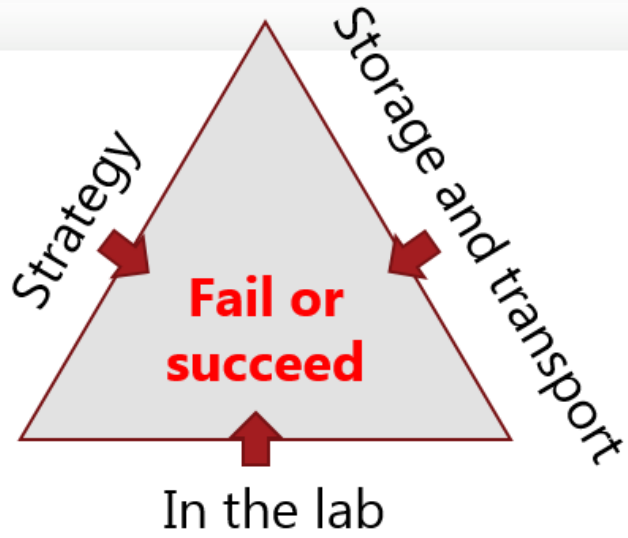
PRRSV detection – the diagnostic triangle!



- **Prior to sampling – decide strategy**
- **In the herd and to the laboratory**
- **In the laboratory**

	In the herd	
	Volume of sample	
	Time and temperature during sampling	
	Freeze-thaw methods	
	Cross contaminations	
	Storage temperature and time	
	Temperature during transport	
	Information to the lab!	

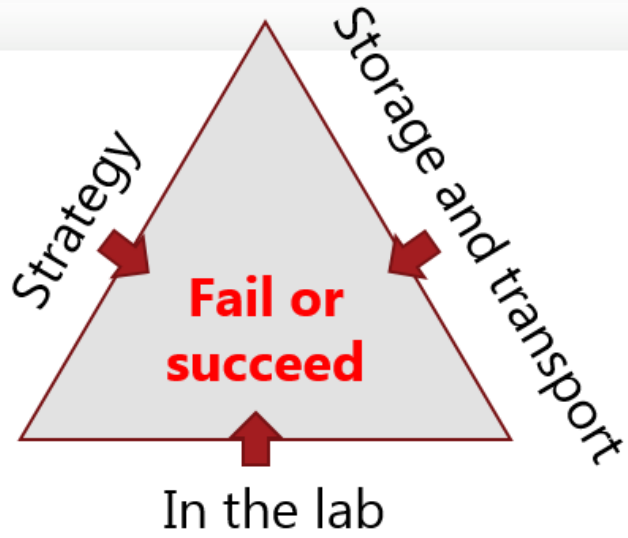
PRRSV detection – the diagnostic triangle!



- **Prior to sampling – decide strategy**
- **In the herd and to the laboratory**
- **In the laboratory**

		In the Lab
		Validation of RNA extraction
		Test for inhibition
		Detection limit and linear range
		Ring trials – benchmark to other labs
		Consequence of pooling
		Communication of border-line test results

PRRSV detection – the diagnostic triangle!



- **Prior to sampling – decide strategy**
- **In the herd and to the laboratory**
- **In the laboratory**

Strategy	In the herd	In the Lab
What is the goal and budget?	Volume of sample	Validation of RNA extraction
Consequence of false negatives	Time and temperature during sampling	Test for inhibition
No of samples	Freeze-thaw methods	Detection limit and linear range
Type of samples	Cross contaminations	Ring trials – benchmark to other labs
Strategy (random, targeted,..)	Storage temperature and time	Consequence of pooling
Interval between samplings	Temperature during transport	Communication of border-line test results
Number of sample times	Information to the lab!	

Lots of data in papers and from experienced wizards

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Effect of pooling family oral fluids on the probability of PRRSV RNA detection by RT-rtPCR

Onyekachukwu H. Osemeke [a], Eduardo de Freitas Costa [c], Marcelo N. Almeida [a], G[illegible] Trevisan [a], Arka P. Ghosh [b], Gustavo S. Silva [a], Daniel C.L. Linhares [a,*]

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A comparison of three sampling approaches for detecting PRRSV in suckling piglets

M.N. Almeida [a,*], M. Zhang [b], W.A.L. Lopez [c], C. Vilalta [d], J. Sanhueza [e], C.A. Corzo [f], J. J. Zimmerman [a], D.C.L. Linhares [a]

[a] Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, 50011, USA

veterinary sciences

Brief Report

PRRSV Detection by qPCR on Serum Samples Collected in Due-to-Wean Piglets in Five Positive Stable Breeding Herds Following a Sow Mass Vaccination with a Modified Live Vaccine: A Descriptive Study

Arnaud Lebret [1,2,*], Valérie Normand [1,2], Charlotte Teixeira Costa [2], Ingrid Messager [3], Pauline Berton [1], Mathieu Brissonnier [1], Théo Nicolazo [2] and Gwenaël Boulbria [1,2]

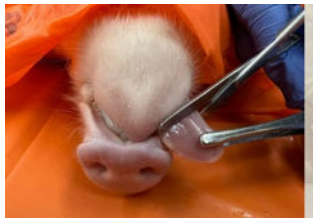
MDPI

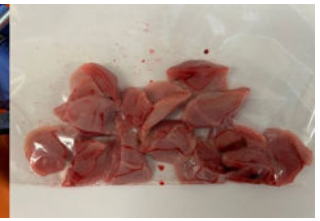
National PRRS REDUKTION

Strategy: Goal and sample type

Focus today

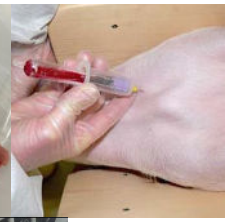
Gestation





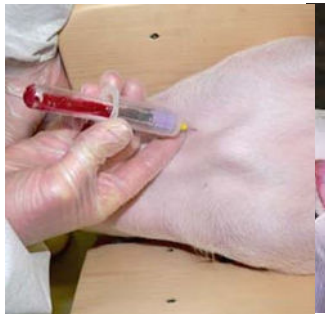
- TTS

Farrowing unit



- TTS from dead pigs
- PF
- OF/FOF
- Serum

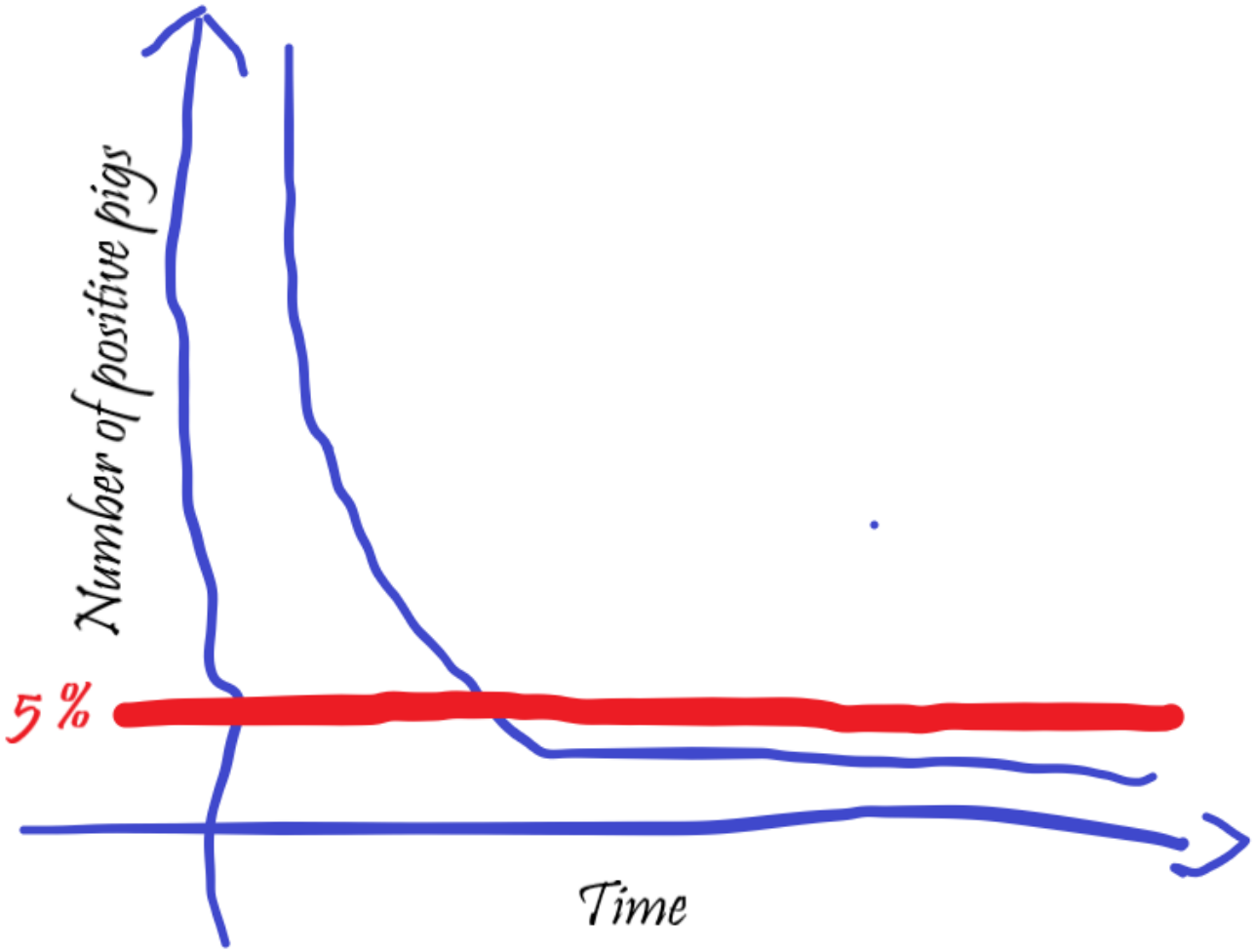
Nusery pigs



- OF/FOF
- Serum
- (TTS from dead pigs)

From Nicolai Weber LFG with permission

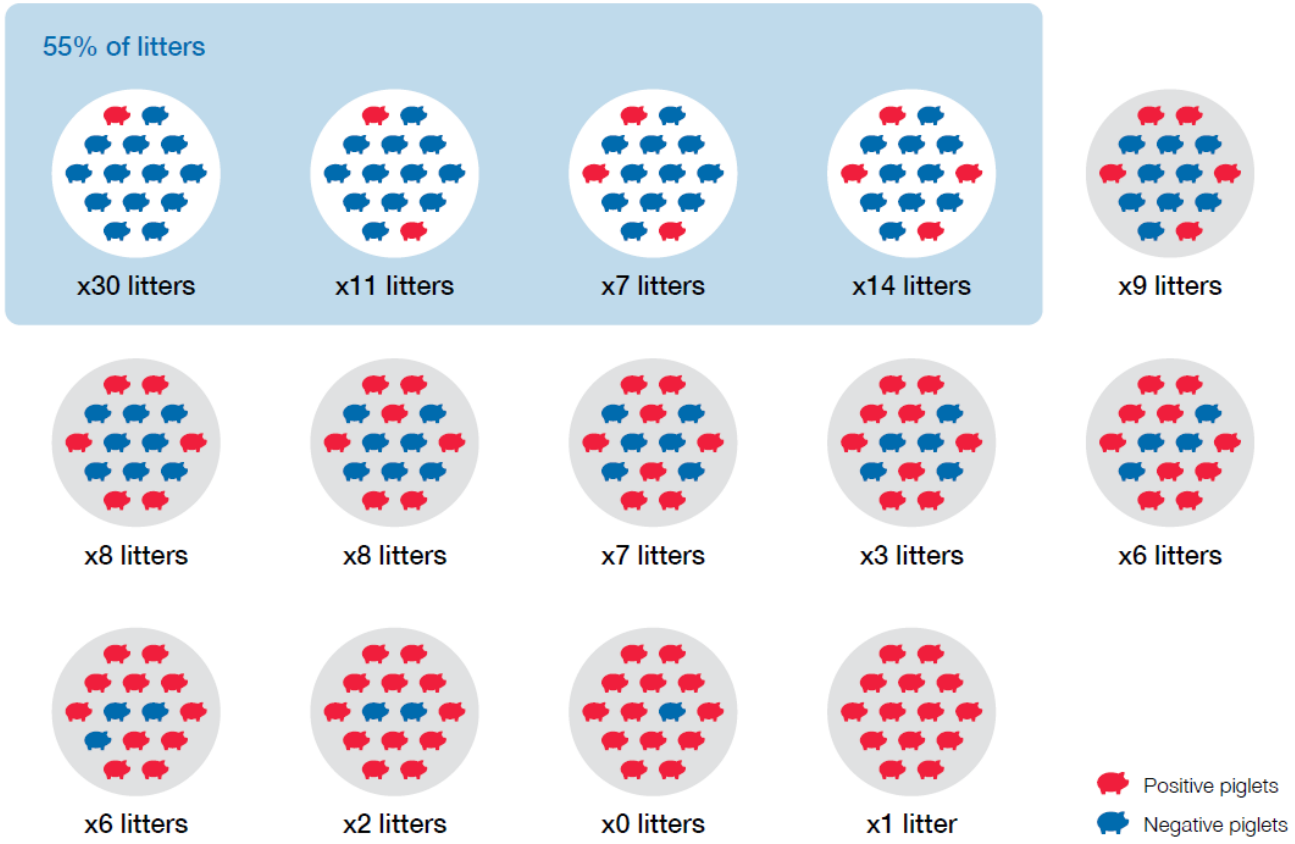
Prevalence of positive weaning age pigs over time during elimination



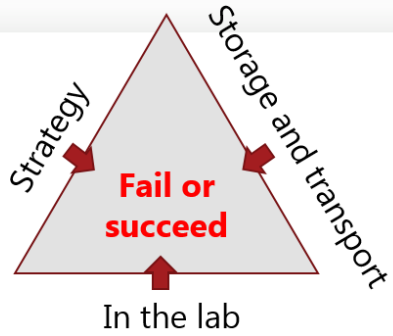
And they are not evenly distributed

Figure 7. **Number of PRRSv-viremic piglets in breeding herd.**

A cross-sectional study was performed in **12 breed-to-wean sow farms** in which serum samples (n = 4510) were collected from all piglets in selected litters (n = 422) in **23 farrowing rooms** and tested individually for PRRSv RNA. In total, **112 litters were tested positive.** This image below shows how PRRSv was distributed in the these positive litters.



The diagnostic paradox



To be sure that you detect the few positive pigs you need to:

First: Sample at least one of the positive pigs

AND!

Next: Detect the virus RNA in that sample

What can possible go wrong

1: Sample at least one of the positive pigs

Challenge: Too expensive to collect and test many serum samples

Prevalence (%)	# Serum samples
~9	30
~5	60
~3	90
~2	120
~1	240
~0.5	400

1: Sample at least one of the positive pigs

Challenge: Also many FOF samples are needed when the prevalence is low

Global PRRS Solutions

When a litter is PRRSv-positive, how many piglets (within the litter) are usually positive?

And how many samples do I need to collect to detect PRRSv at different prevalences (95% confidence)?

Table 2. Number of serum and FOF samples to achieve 95% confidence to detect PRRSv at different prevalence scenarios.

Prevalence (%)	# Serum samples	# FOF samples
~9	30	5
~5	60	7
~3	90	10
~2	120	15
~1	240	30
~0.5	400	40

Example: 90 serum samples or 10 FOF, per air space, is needed to achieve 95% confidence to detect at least 1 sample positive when prevalence is 3% or higher.

Note the high sample size required for serum compared to FOF for all prevalence scenarios. One of the reasons is that FOF includes biological sample from multiple animals.

Solution: Pooling of samples! – but watch out!

Henry
Osemeke
Field Epi - ISU

Probability that you sample the positive pig

Number of pigs/pools per test-pool

Probability that the sample test positive

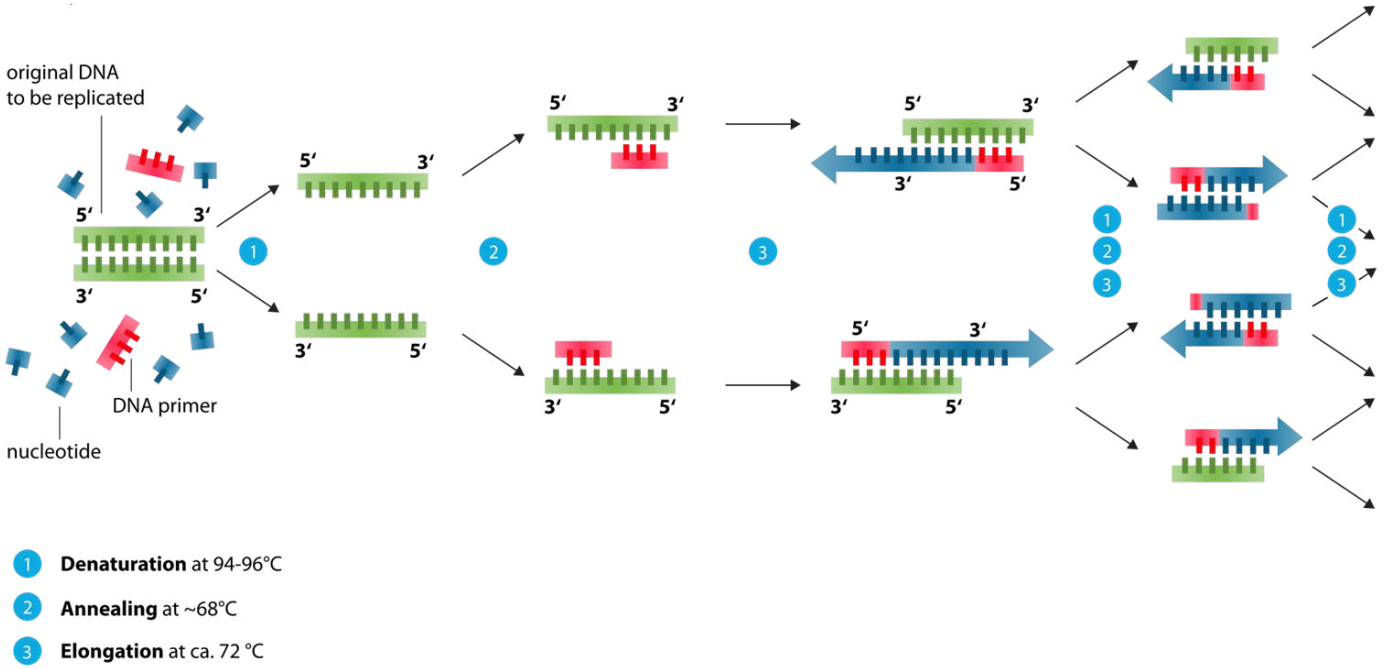
- Dillution effect

Number of pigs/pools per test-pool

From Nicolai Weber LFG with permission

1: Sample at least one of the positive pigs

Sidestep: PCR basic

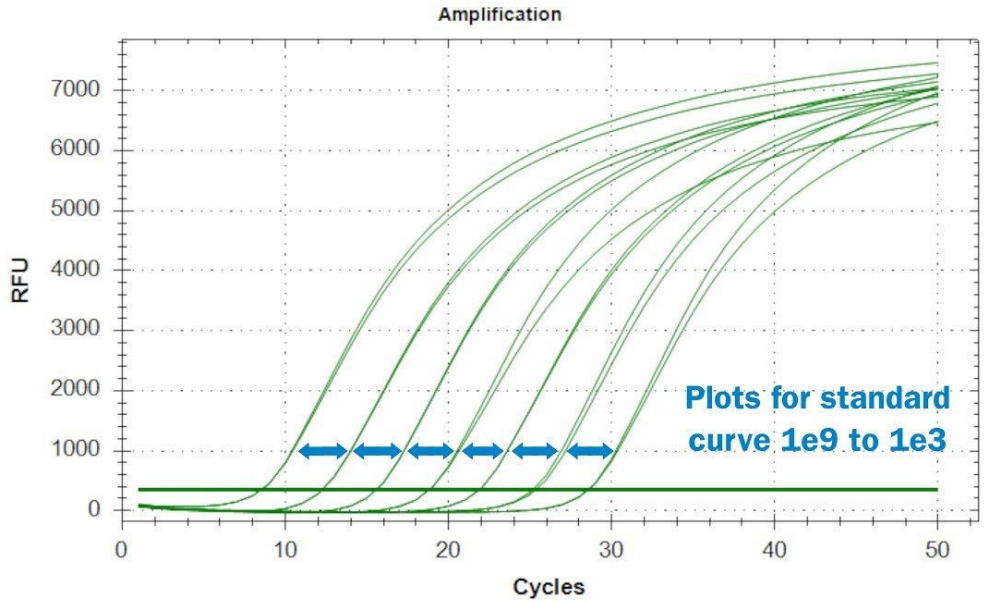


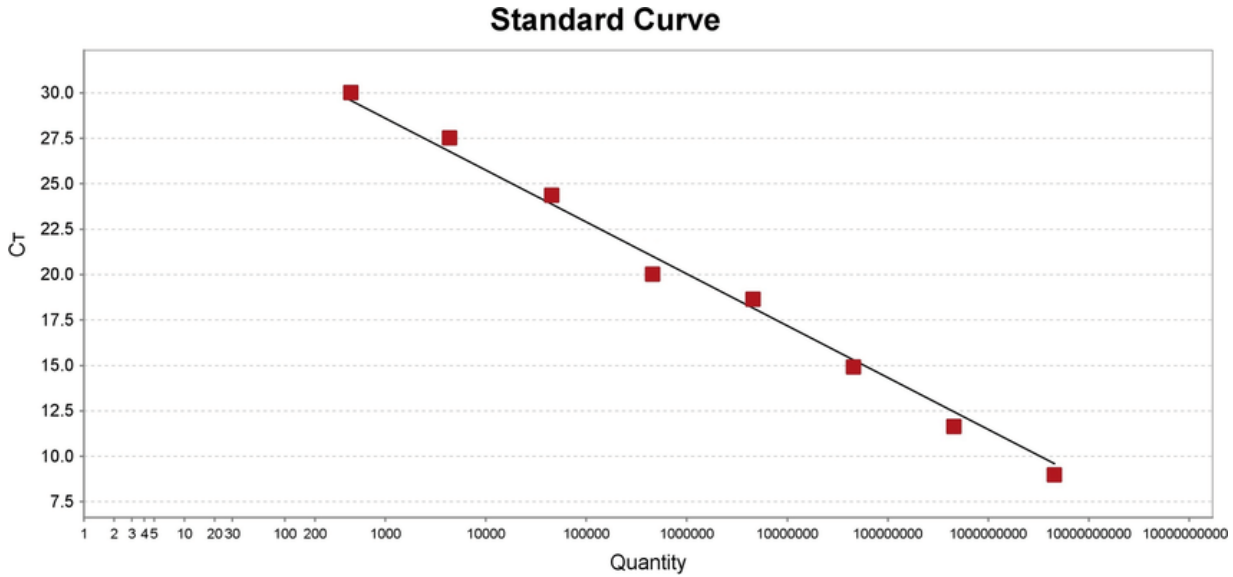
1. **Denaturation** at 94-96°C
2. **Annealing** at ~68°C
3. **Elongation** at ca. 72 °C

CYCLE	AMOUNT OF DNA
	100% EFFICIENCY
0	1
1	2
2	4
3	8
4	16
5	32
6	64
7	128
8	256
9	512
10	1,024
11	2,048
12	4,096
13	8,192
14	16,384
15	32,768
16	65,536
17	131,072
18	262,144
19	524,288
20	1,048,576
21	2,097,152
22	4,194,304
23	8,388,608
24	16,777,216
25	33,554,432
26	67,108,864
27	134,217,728
28	268,435,456
29	536,870,912
30	1,073,741,824

= 1.4 trillion tonnes = 2000 times the yearly global grain production!

Sidestep: PCR basic





For each 10-fold dilution the Ct value increases with 3.3
(if the test has an efficacy of 100 %)

1: Sample at least one of the positive pigs

Serum versus oral fluids (OF) or family oral fluids (FOF)

veterinary sciences

MDPI

Brief Report

PRRSV Detection by qPCR on Serum Samples Collected in Due-to-Wean Piglets in Five Positive Stable Breeding Herds Following a Sow Mass Vaccination with a Modified Live Vaccine: A Descriptive Study

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Table 2. Comparison of PRRSV-1 RT-qPCR detection in serum and FOF from litters of due-to-wean piglets.

		Serum		
		NEG	POS	Total
FOF	NEG	103	7	110
	POS	3	6	9
	Total	106	13	119

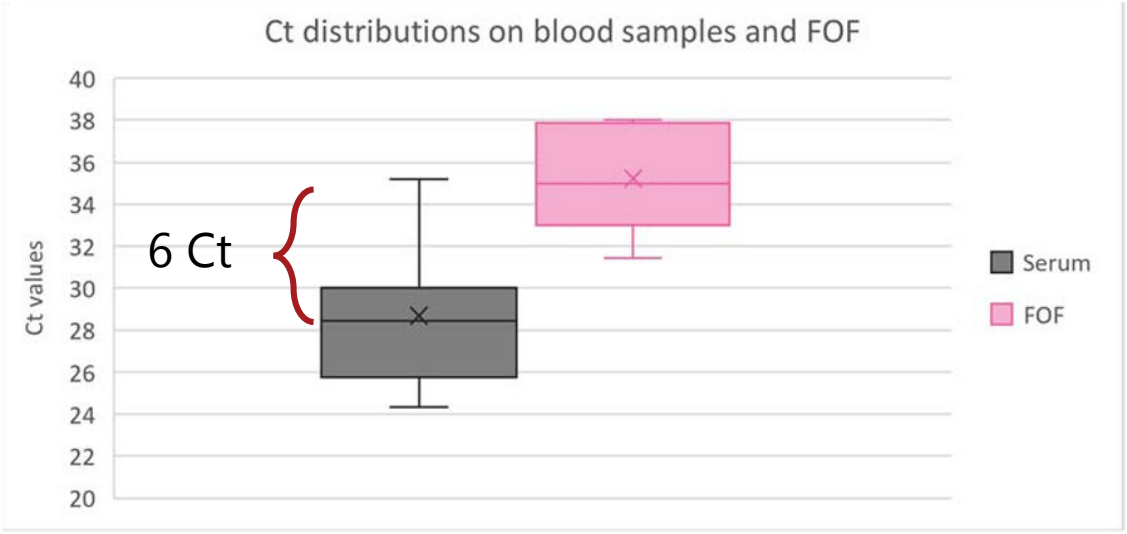
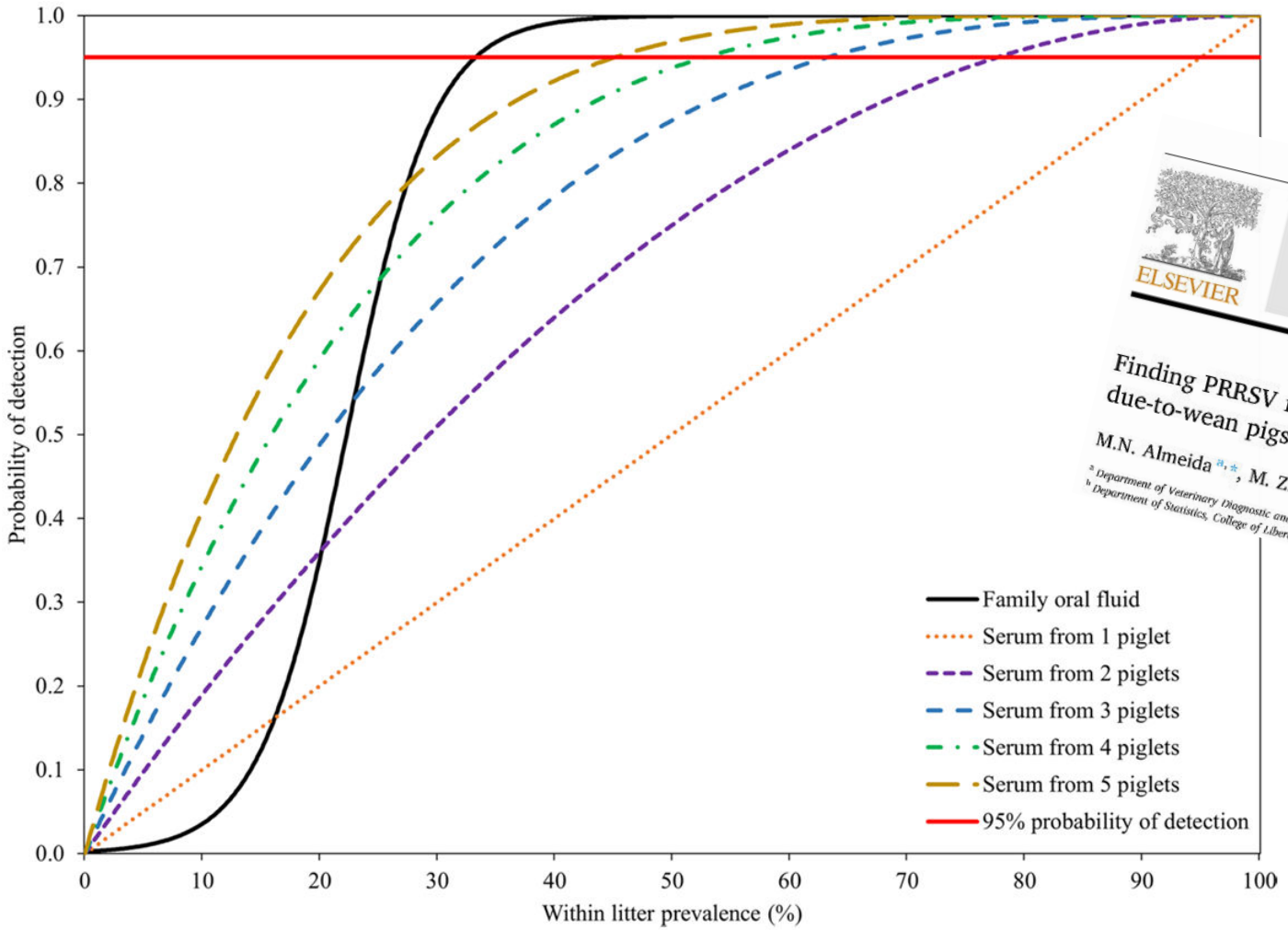
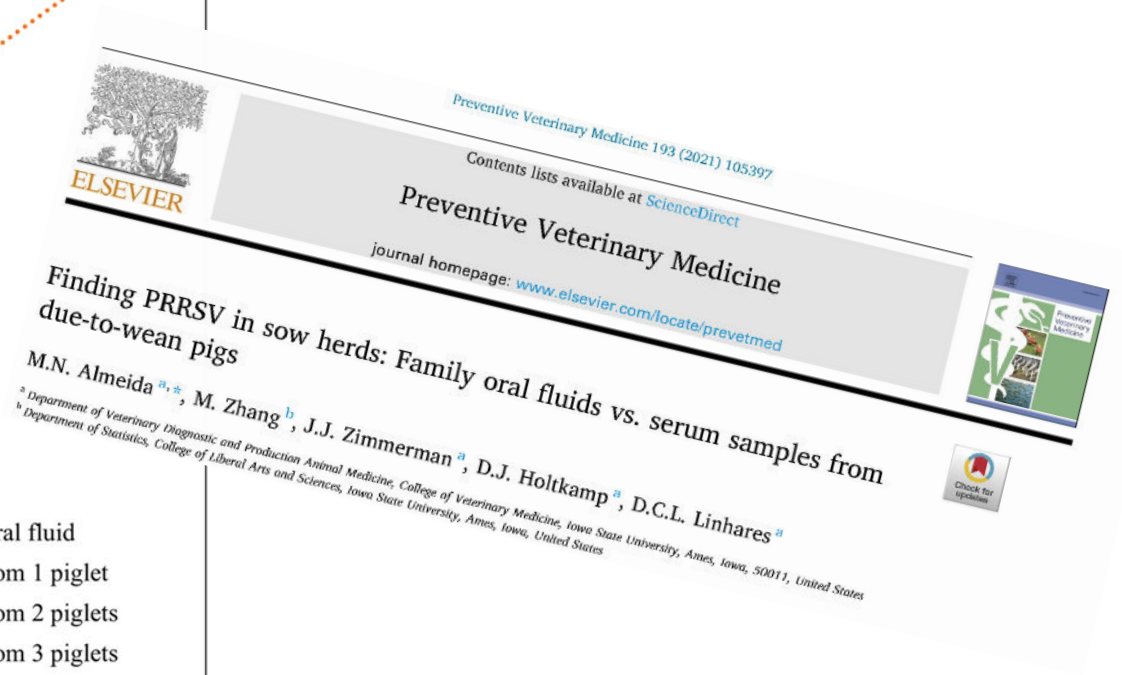


Figure 1. Distribution of cycle threshold (Ct) values for detection of PRRSV-1 from positive blood samples and positive family oral fluids (FOF) samples using RT-qPCR. Boxplots show median, quartiles, minimum and maximum values.

BUT! FOF has been shown to be reliable for mass testing

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ELSEVIER

Finding PRRSV in sow herds: Family oral fluids vs. serum samples from due-to-wean pigs

M.N. Almeida [a,*], M. Zhang [b], J.J. Zimmerman [a], D.J. Holtkamp [a], D.C.L. Linhares [a]
[a] Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, 50011, United States
[b] Department of Statistics, College of Liberal Arts and Sciences, Iowa State University, Ames, Iowa, United States

Fig. 1. Probability of PRRSV RNA detection using family oral fluids (FOF) according to number of viremic piglets within a litter.

1: Sample at least one of the positive pigs

Back to pooling – success depends on the Ct value of the positive pig!

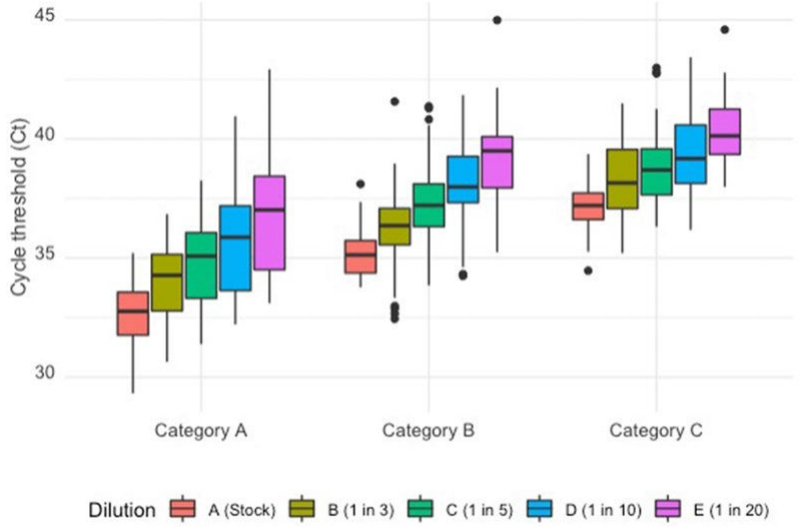
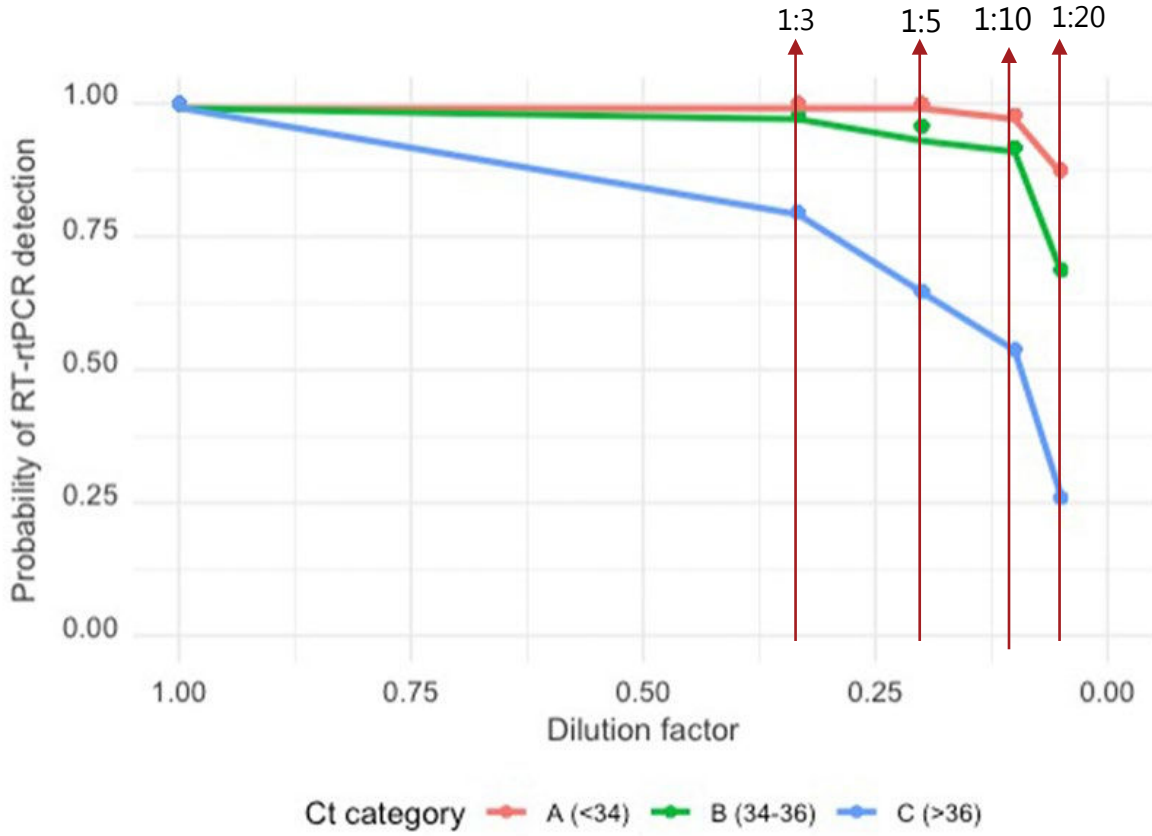


Fig. 1. PRRSV RT-rtPCR Ct changes per dilution level for each of the 3 Ct value categories.



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[b] Department of Statistics, Iowa State University, Ames, IA, USA
[c] Department of Epidemiology, Bioinformatics and Animal Models, Wageningen Bioveterinary Research, Lelystad, the Netherlands

The billion dollar question in pooling

How positive is the positive pigs?

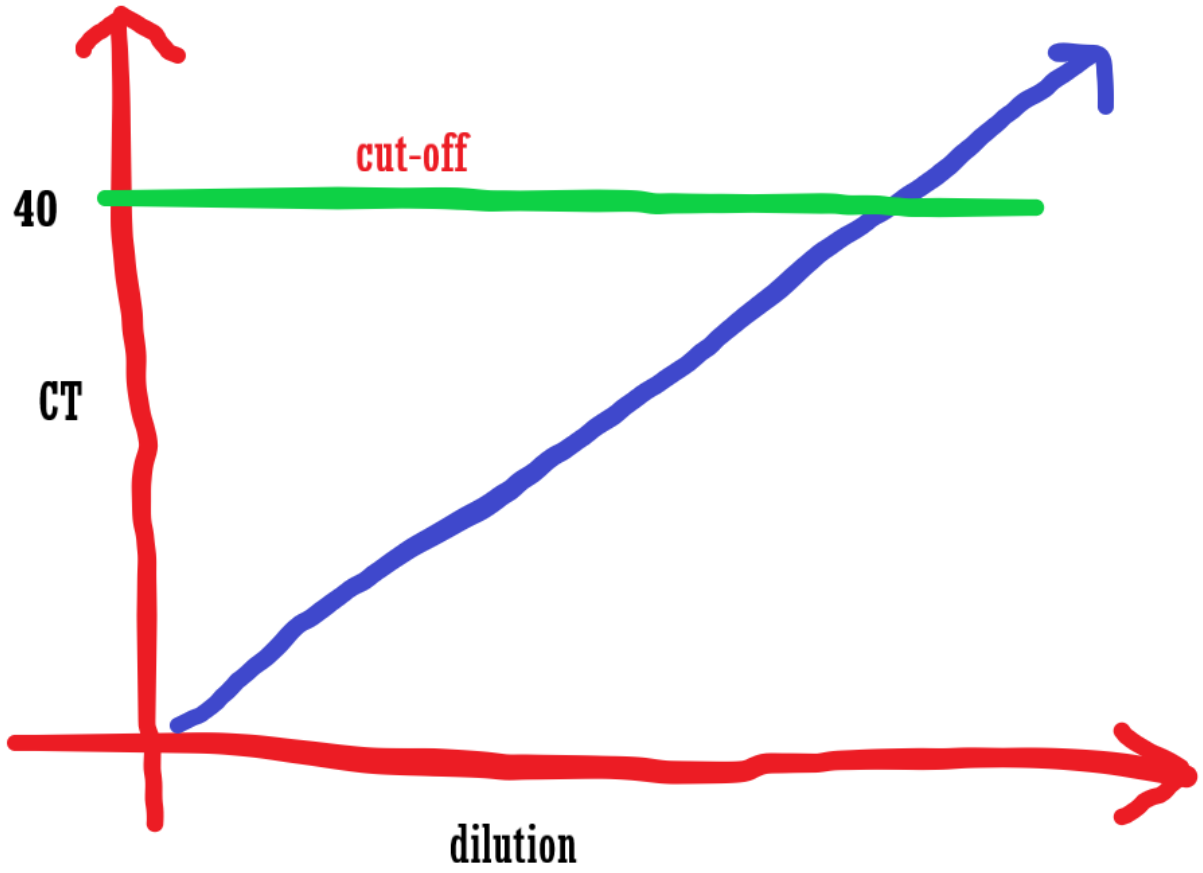


Table 3. Ct values of sera and FOF samples (with batch identification) tested individually, pooled by 3 and pooled by 5 (ND = not done).

SERA					FOF				
Sample Identification	Batch	Ct Individual	Ct Pool 1:3	Ct Pool 1:5	Sample Identification	Batch	Ct Individual	Ct Pool 1:3	Ct Pool 1:5
Serum-1	1	24.9	28	28.9	FOF-1	1	31.4	32.5	34.8
Serum-2	1	24.3	28	28.9	FOF-2	2	33	>40	>40
Serum-3	1	25.5	28.3	29.4	FOF-3	2	38	>40	>40
Serum-4	2	26	28.4	29.2	FOF-4	2	38	>40	>40
Serum-5	2	33	34.4	37	FOF-5	2	35	35.4	>40
Serum-6	2	28	31.2	32	FOF-6	2	33	>40	>40
Serum-7	2	30	33.8	34.9	FOF-7	3	36.8	>40	>40
Serum-8	2	30	32.8	34.3	FOF-8	3	37.8	>40	>40
Serum-9	2	30	>40	>40	FOF-9	4	34.2	>40	>40
Serum-10	2	29	ND	ND					
Serum-11	3	35.2	>40	>40					
Serum-12	3	28.4	30.7	31.6					

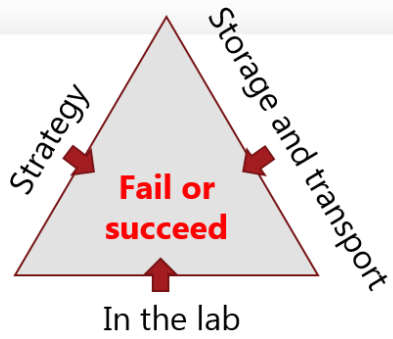
Lebret et al. 2023

Limited data from Denmark

- Ct values on samples tested at Kjellerup and SSI (no info on pool size)

Spyt	SSI	VLK
Ct min	28	32
Ct max	36	37
Ct middel	35	32
Ct median	35	32
PF		
Ct min	22	23
Ct max	42	37
Ct middel	34	31
Ct median	35	32
serum		
Ct min	14	25
Ct max	40	36
Ct middel	29	31
Ct median	29	36

The diagnostic paradox



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Next: Detect the virus RNA in that sample

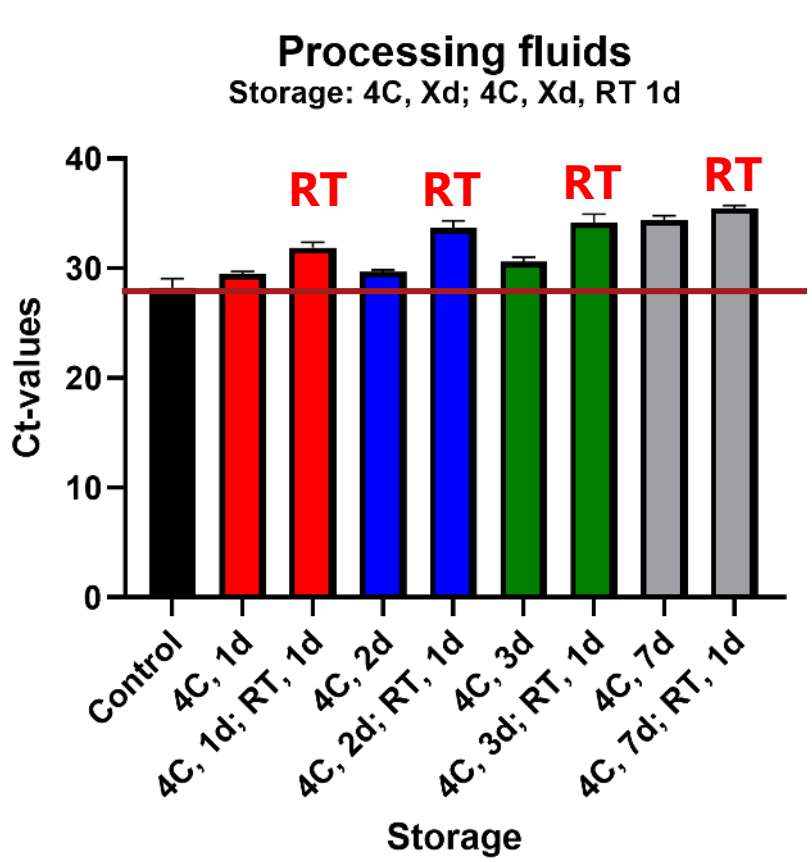
What can possible go wrong

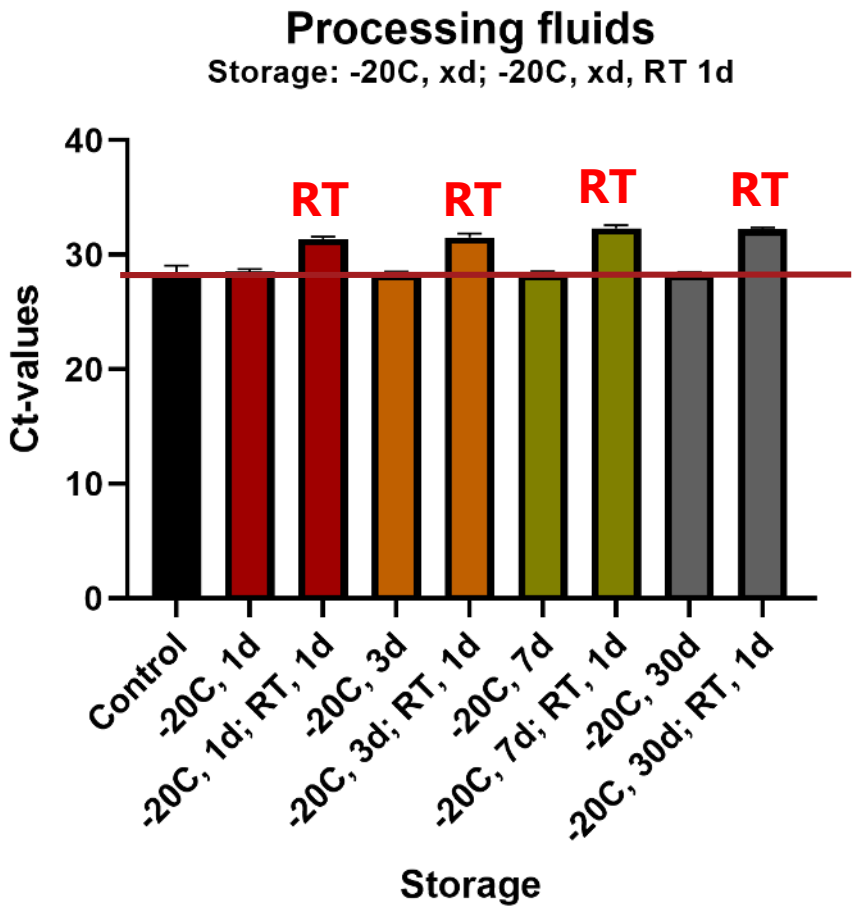
In the herd
Volume of sample
Time and temperature during sampling
Freeze-thaw methods
Cross contaminations
Storage temperature and time
Temperature during transport
Information to the lab!

Storage of samples

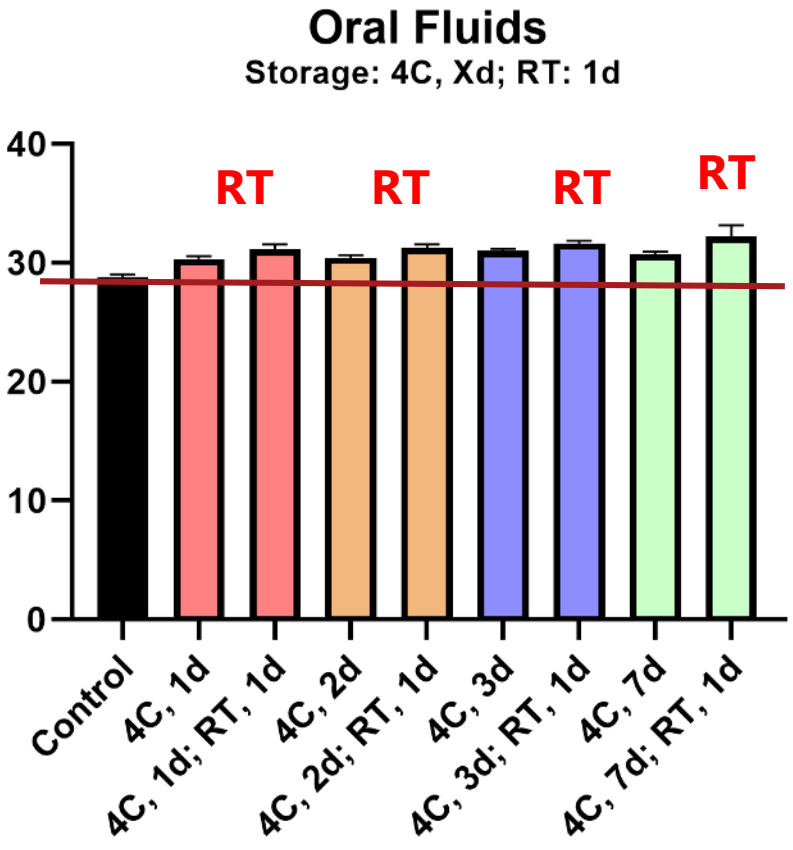
- PF, OF and serum spiked with an PRRSV-1 virus isolate
- **Storage combinations**
- 4C for 1, 2, 3 and 7 days
- 4C i 1, 2, 3 for 7 days + at Room Temperature (RT) 1 day
- -20C for 1, 3, 7 and 30 days
- -20C for 1, 3, 7 and 30 days + RT 1 day
- **Only serum**
- RT for 1, 2, 3 and 7 days
- -80C for 1, 3, 7 and 30 days

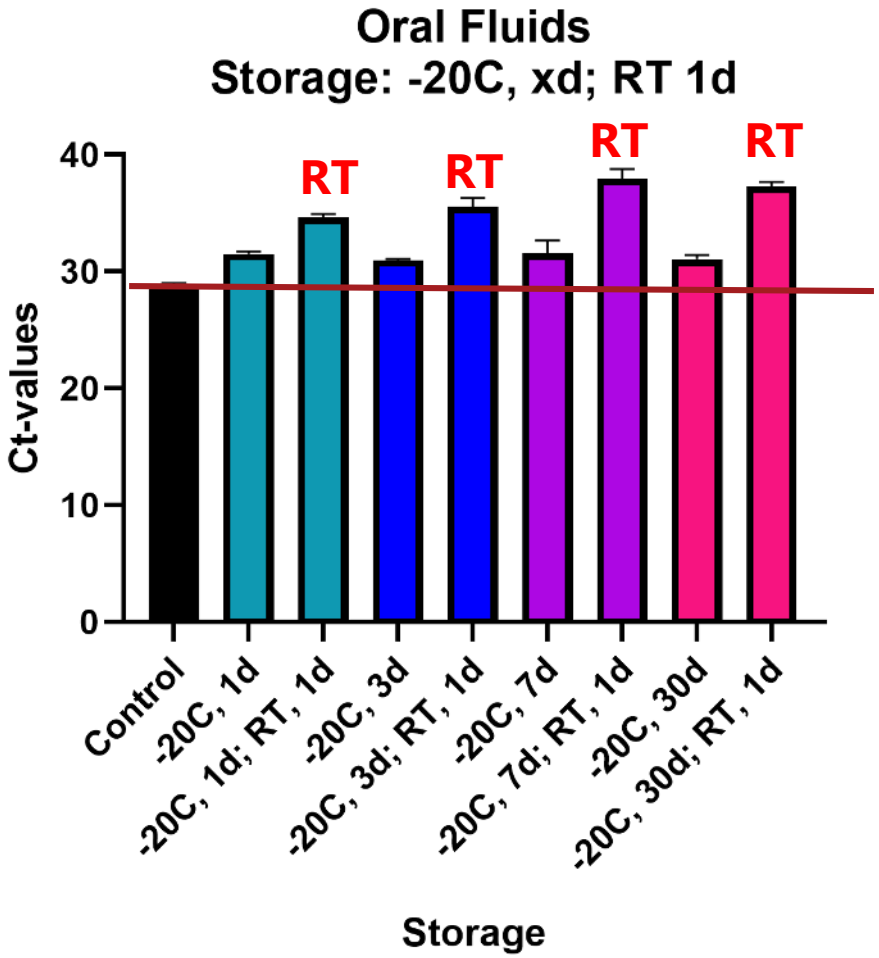
Results processing fluids (4C and -20C)



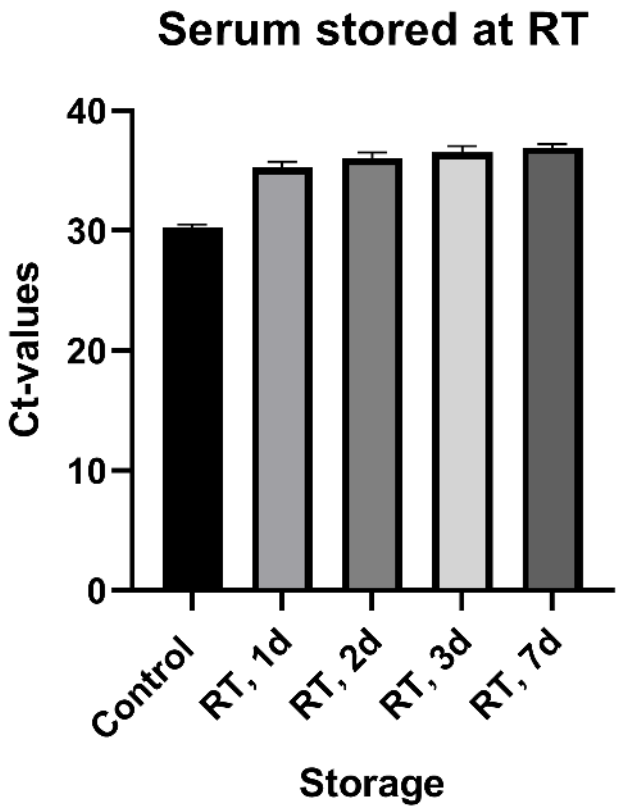


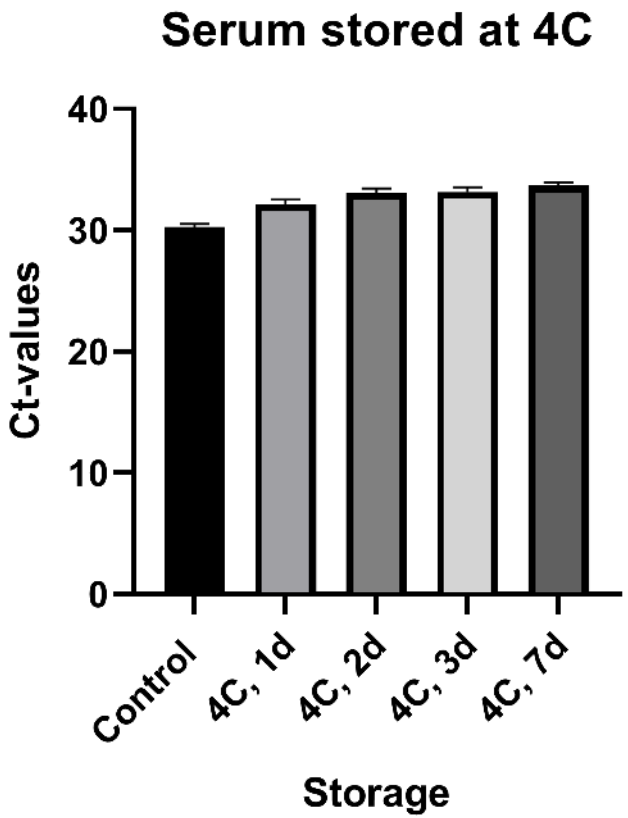
Results oral fluids (4C og -20C)

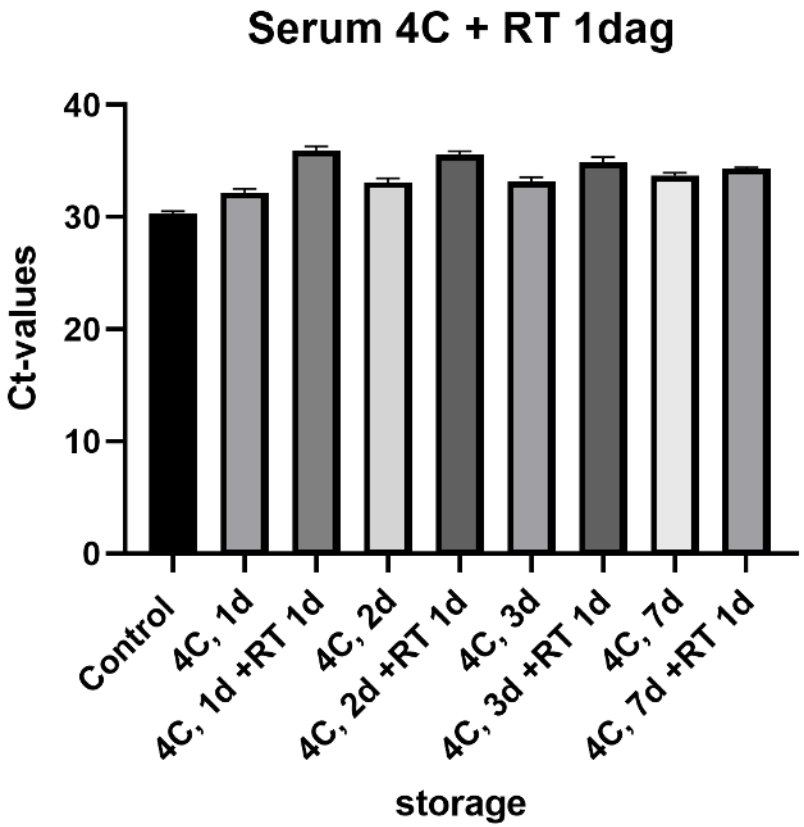




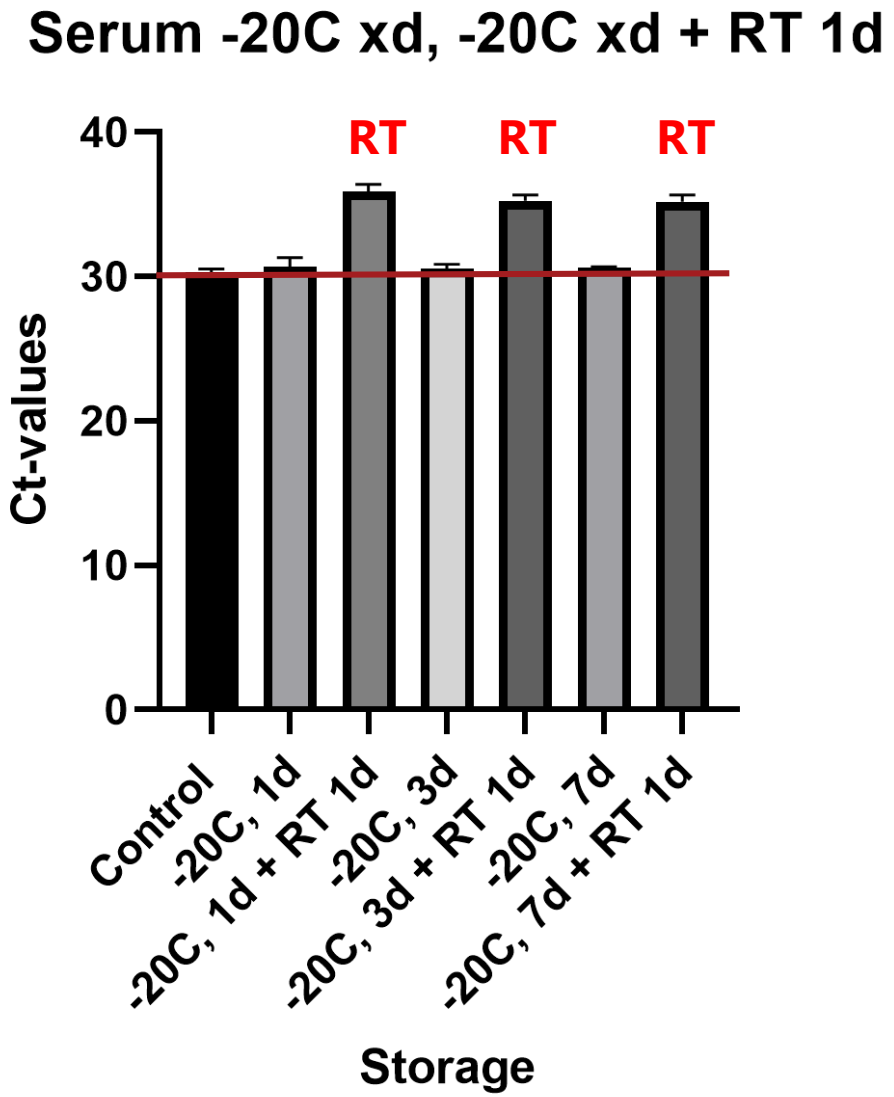
Results serum



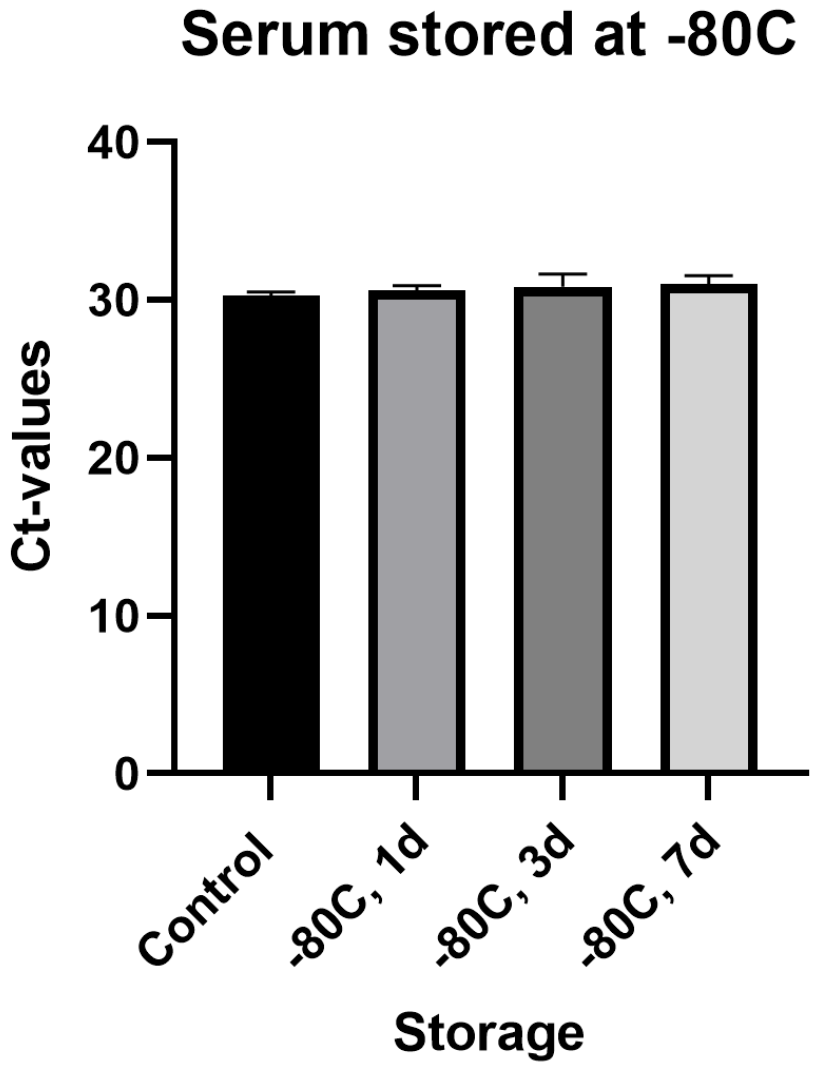


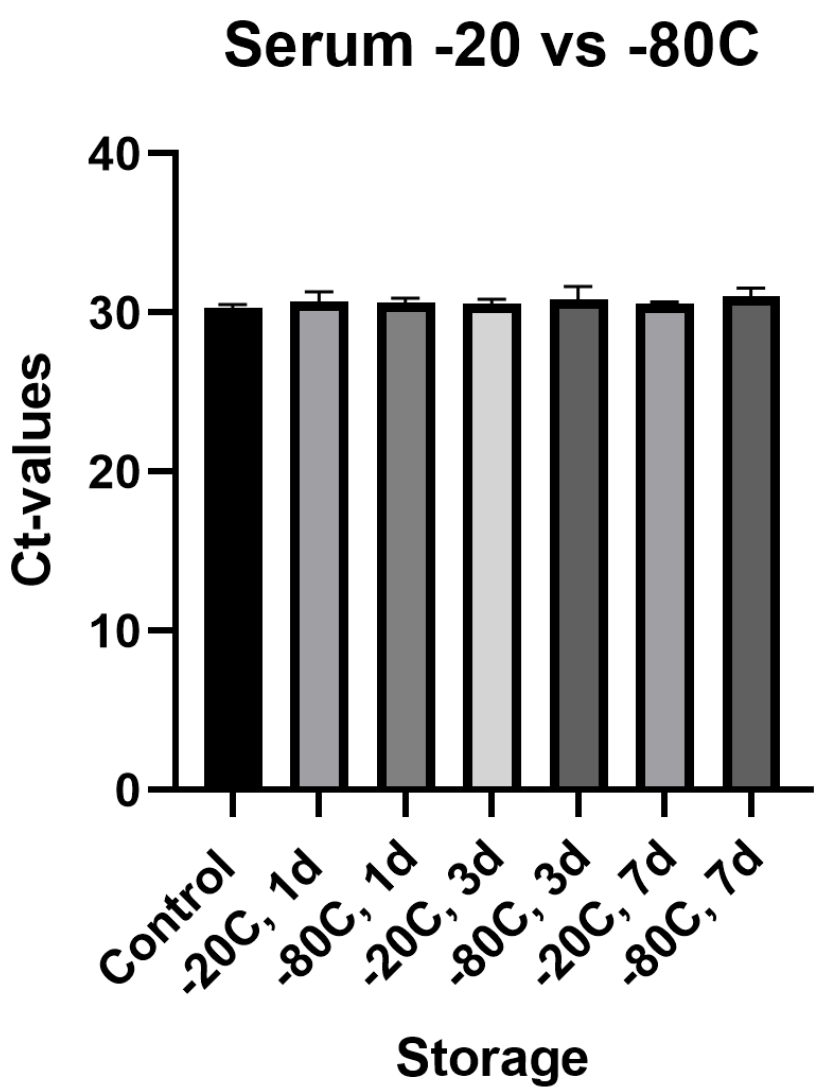


Results serum (-20 C)



Results serum (-80C)





Conclusion on sample storage

- The amount of virus in blood samples stored for one day at room temperature decreased significantly
- For **PF and OF, the drop was even greater**!
- All samples should therefore be kept refrigerated **ASAP** after collection – and preferable also during sampling
- PF bags and OF bags should either be sent frozen in a cooling bag or left in the **refrigerator for thawing**
- Storage at 4C can be accepted for shorter periods - no more than 1 day
- Storage at -20C vs -80C showed no difference in the period tested
- 1-2 freeze-thaw cycles do not have significant effect

Micowave and heated floors for thawing is absolutely NO GO!!!

A theoretical example for a worse case scenario

- Loss in Ct compared to immediate test of blood from the positive pig
  - OF/FOF instead of serum: 6 Ct
  - Pooling 1:10 3 Ct
  - Wrong storage in herd: 2 Ct
  - Thawing at RT for 1 day: 4 Ct
  - **Total** **15 Ct**

- **Thus, the viraemic pig should have a viral serum load corresponding to a Ct value of 25 or below to test positive (at Ct 40 as cut off)**

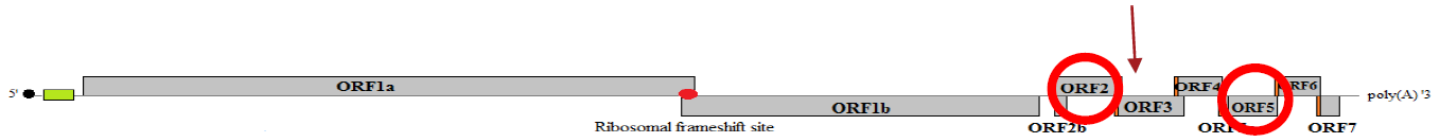
Sum up

- Prioritize intensive sampling when the consequence are high
- Many samples are needed at low prevalences
  - Use of pooling increase the probability of sampling the positive pig but comes with a price!
  - The risk of false negative conclusion of weaner pig status can be mitigated by:
    - Correct storage of the samples during and after sampling; and during transport
    - Repeat the sampling according to the recommendations – at least four consecutive negative samplings
    - Make sure to cover all sections – the viraemic pigs may cluster
    - Combine serum/FOF with test of dead animals (TTS) – relatively cheap

PRRSV sekventering

- Vi tilbyder PRRSV-sekventering af diagnostiske prøver (SSI, Kjellerup) gratis (finansieret af Svineafgiftsfonden)
- Vi vil gerne have lidt baggrunds information: kliniske tegn, seneste ændringer fra neg ->pos, vaccinationsstatus mm

- PRRSV-1: partiel ORF2 and ORF5 som udgangspunkt



- PRRSV-2: ORF5
- ORF2-7 og fuld genom på udvalgte prøver

PRRSV laboratorie svar

STATENS SERUM INSTITUT | KØBENHAVNS UNIVERSITET

Svarrapport status

Sagsnr. 2023-04530

Resultat kommentar

Den sekventerede prøve er 96,64 % identisk i partiel ORF2 (684 nukleotider) og 96,37 % identisk i ORF5 til vaccinestammen brugt i Porcilis MLV (MT311646).

Konklusion:
Den sekventerede virus tilhører Porcilis-like clusteret.

I tabellen er angivet prøvens lighed i % til udvalgte PRRSV-1 vaccinestammer i ORF5:

Vaccine:	Unistrain	Porcilis PRRS	Suvaxyn PRRS
Stamme:	Amervac	DV	96V198
Genbank #:	GU067771	MT311646	LQ787782
Prøve 2 :	92,57	96,37	87,79

Kopi til:

Resultat kommentar 2023-06412

Den sekventerede prøve er 95,52% identisk i ORF5 til Ingelvac MLV vaccinestammen (EF484033).

PRRSV-1 ORF5 fylogenetisk træ

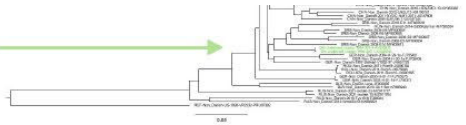
Porcilis like

Porcilis MLV

PRRSV Sekvens Hjemmeside

prrsv.dk

Udefineret cluster



Thank you for your attention

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  - SEGES Innovation
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  - SSI
    - Charlotte Hjulsager