GF-TADs
GLOBAL FRAMEWORK FOR THE PROGRESSIVE CONTROL OF TRANSBOUNDARY ANIMAL DISEASES

Food and Agriculture Organization of the United Nations

WORLD ORGANISATION FOR ANIMAL HEALTH

ASF laboratory diagnostics and rapid tests

Practices, lessons learnt and perspectives

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Why do we need laboratory diagnosis?

Differentiation of African and classical swine fever is impossible based on clinical signs alone! The same is true for several other differential diagnoses!

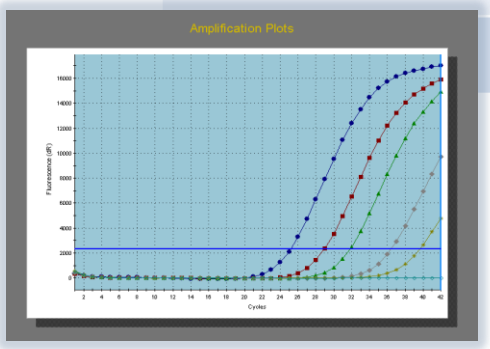
Domestic fattening pigs at the seventh day post inoculation with a highly virulent ASFV strain („Armenia08“)

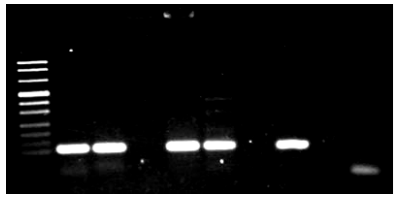
Domestic pigs and wild boar at the seventh day post inoculation with a highly virulent CSFV strain („Koslov“)

Laboratory confirmation is mandatory!

Routinely used detection methods

Infectious virus	Viral antigen	Viral genome	Antibodies
Virus isolation	Staining of cryo-sections	Gel-based PCR	**ELISA**
Haemadsorption test	Antigen ELISA	**Real-time PCR**	Indirect immuno-peroxidase test
(Bioassay)	(Lateral flow assays)	Sequencing	Indirect fluorescent antibody test
		(Direct PCR)	Immunoblot
		(Isothermal methods)	Lateral flow assays

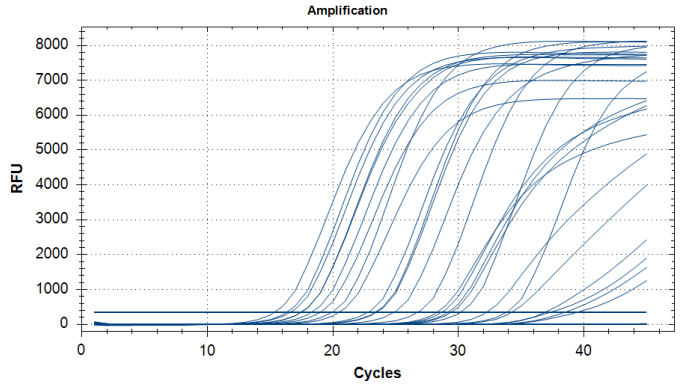




See also: https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.08.01_ASF.pdf

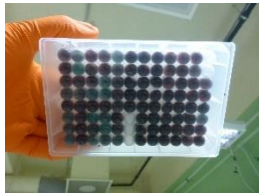
Real-time PCR

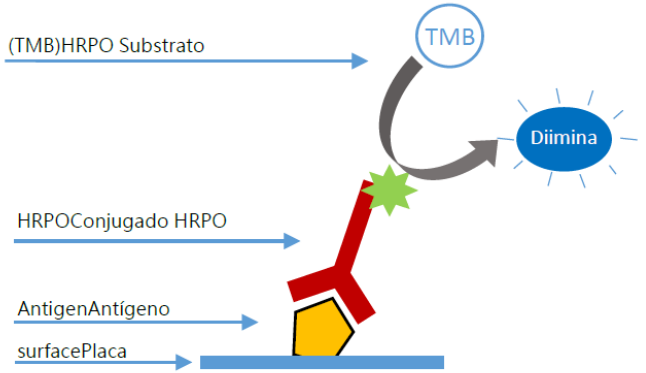
- High sensitivity and specificity
- Real-time PCR can be used with a large variety of sample matrices
- Reliable detection from ~3-4 days post infection (often for 70 to 90 days)
- Rather expensive, requires good equipment
- Commercial tests are available and most are suitable (this is an advantage!)
- Nucleic acid extraction is needed and can be a crucial point
- Prefer tests with internal controls (heterologues or endogenous) that secure reliability of negative results
- High sensitivity means also high risk of contamination!!! Always check plausibility and have back-up systems available (in the lab)
- Back-up systems should target another genomic region and are only used for confirmatory testing



Antibody ELISA

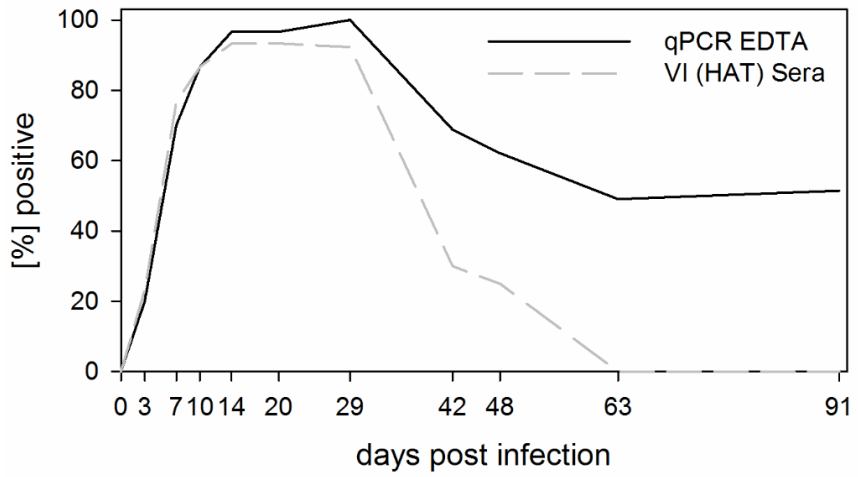
- Detection of an antigen-antibody reaction with labeled antibodies
- Labelling with an enzyme, often horseradish peroxidase
- Measurement of the optical density at a defined wavelength
- Rather qualitative results
- Usually fit for purpose, moderate sensitivity and specificity
- Sample matrix: Serum, Plasma, (filter paper/swab punches, meat juice)
- Reliable detection from ~10 days post infection (for at leat several months)
- Rather low tech, but water quality and optical instruments are crucial
- Commercial tests are available and most are suitable (this is an advantage!)
- Indirect and competitive formats are available, systems use different antigens
- Confirmatory testing is beneficial

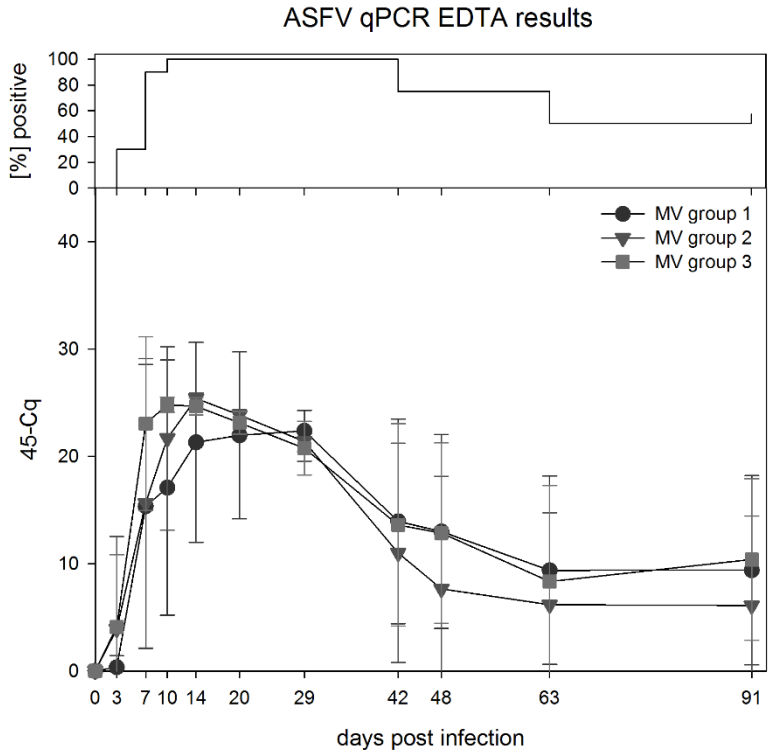




What can we expect to detect?

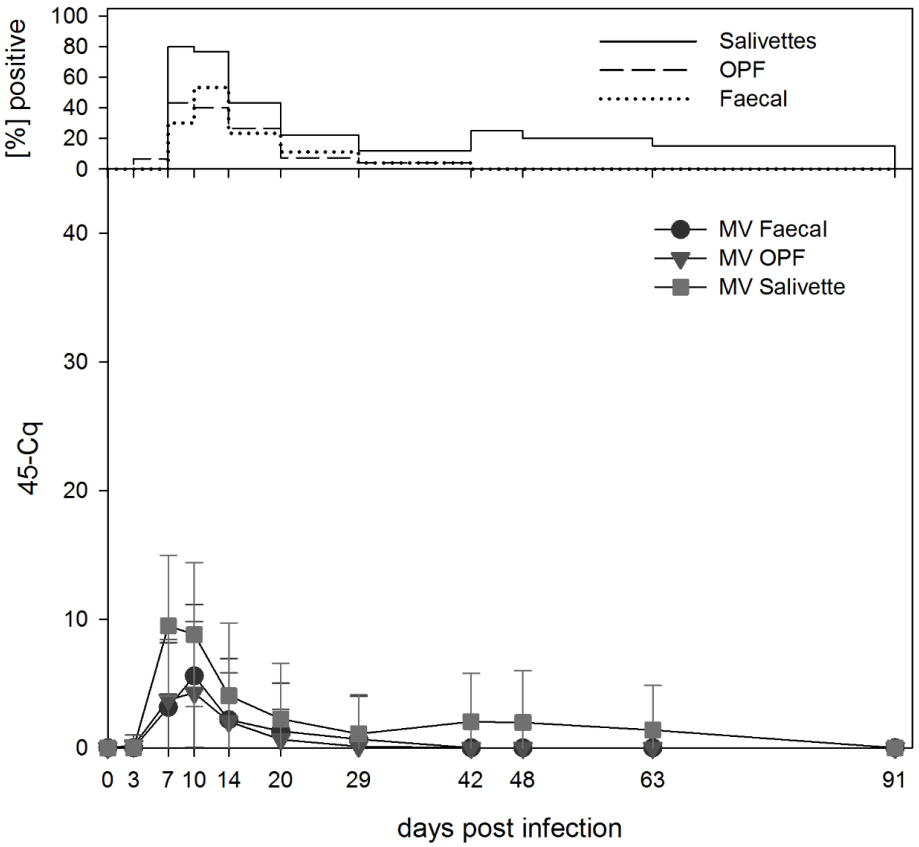
- ASFV is originally an ARBO virus → blood contains high viral loads
- Viraemia is rather long, even in convalescent animals
- However, chose a sick pig to obtain initial samples!
- Viral genome is NOT infectious virus!





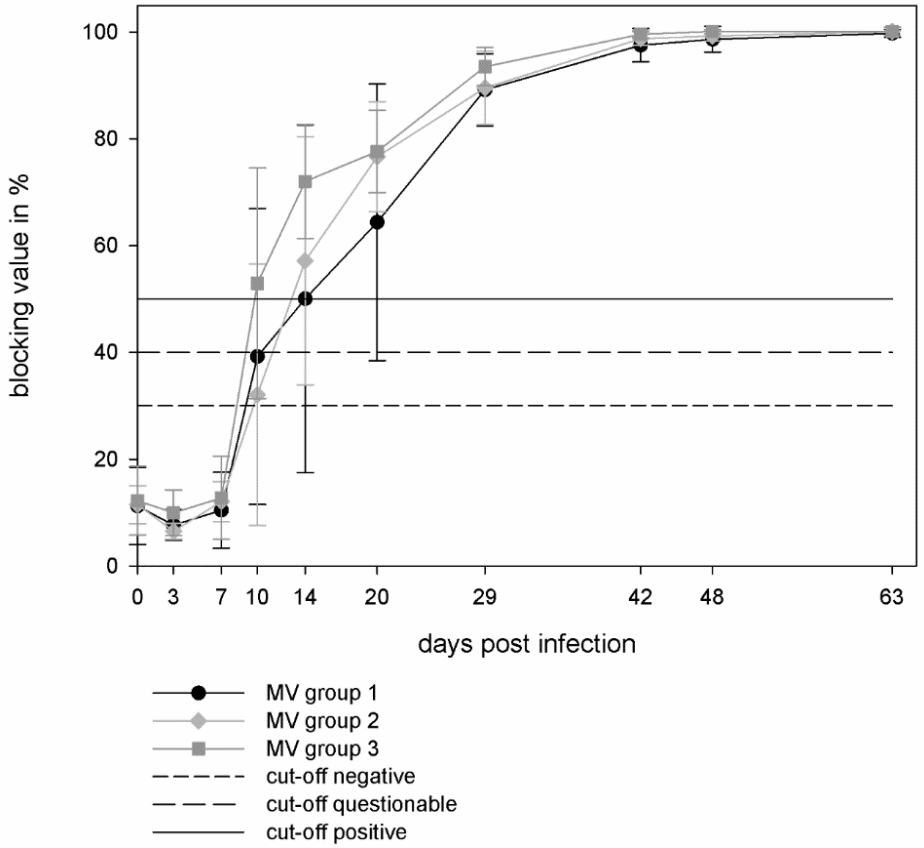
Source: Petrov A, Forth JH, Zani L, Beer M, Blome S. No evidence for long-term carrier status of pigs after African swine fever virus infection. Transbound Emerg Dis. 2018;65(5):1318-1328. doi:10.1111/tbed.12881

What can we expect to detect?



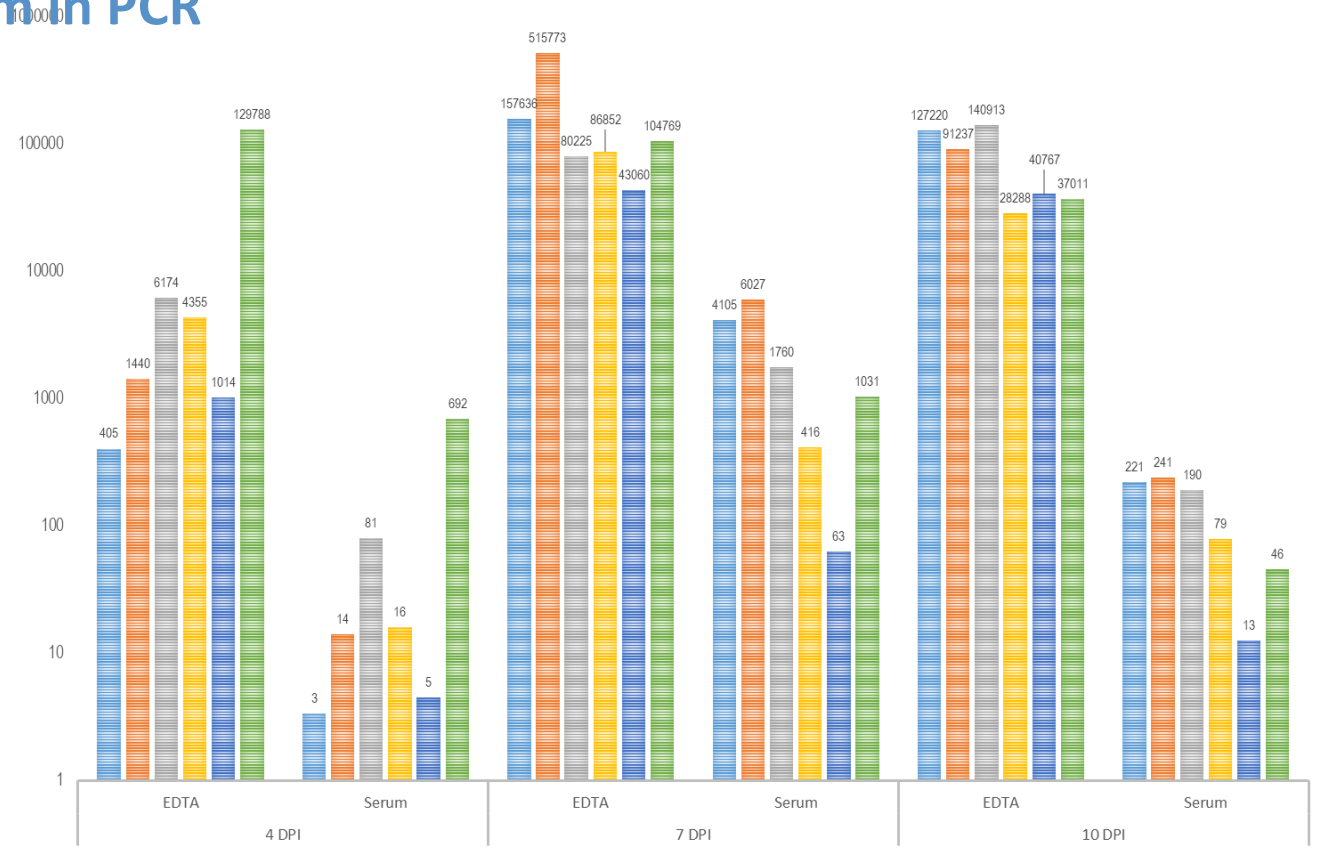
- Shedding with se- and excretions is rather low (suitability of matrices?)
- Swabs may not help with small holdings or individual animals…, they may be „fit for purpose“ in large holdings (herd-based) or when sampling dead animals (see results from Belgium)
- Hemorrhagic signs will aid detection (bloody fecal or oro-pharyngeal swabs)

What can we expect to detect?



- Antibodies appear roughly between days 7 and 14 post infection
- No predictive value for final disease outcome
- Antibodies will tell about the age of infection
- Use antibody detection to establish high risk periods
- Detect infections with strains of lower virulence (that went unnoticed)

EDTA blood vs serum in PCR



Serum contains significantly less viral genome! Be careful with pooling…

Targets in the EU: carcasses...



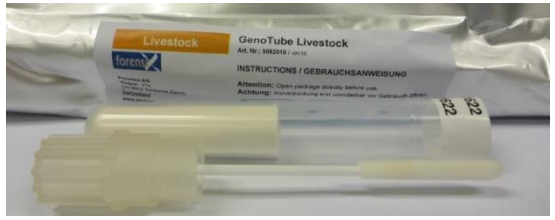


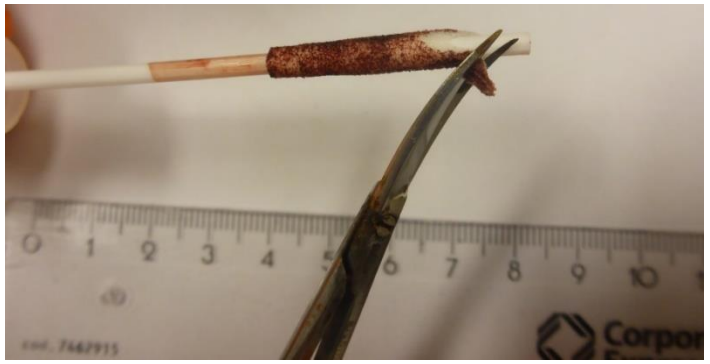


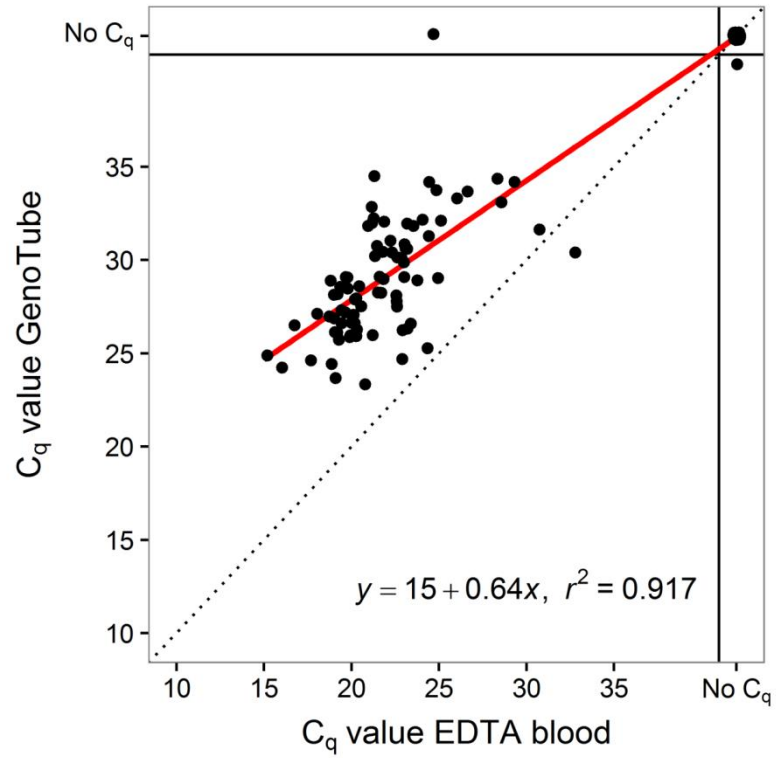

Source: Food and Veterinary Service of Latvia

- Sampling is not easy
- Rotten carcasses are smelly and disgusting…
- Some organs are not available anymore
- Blood (decent fluid) is not available
- Some carcasses are just skeletons

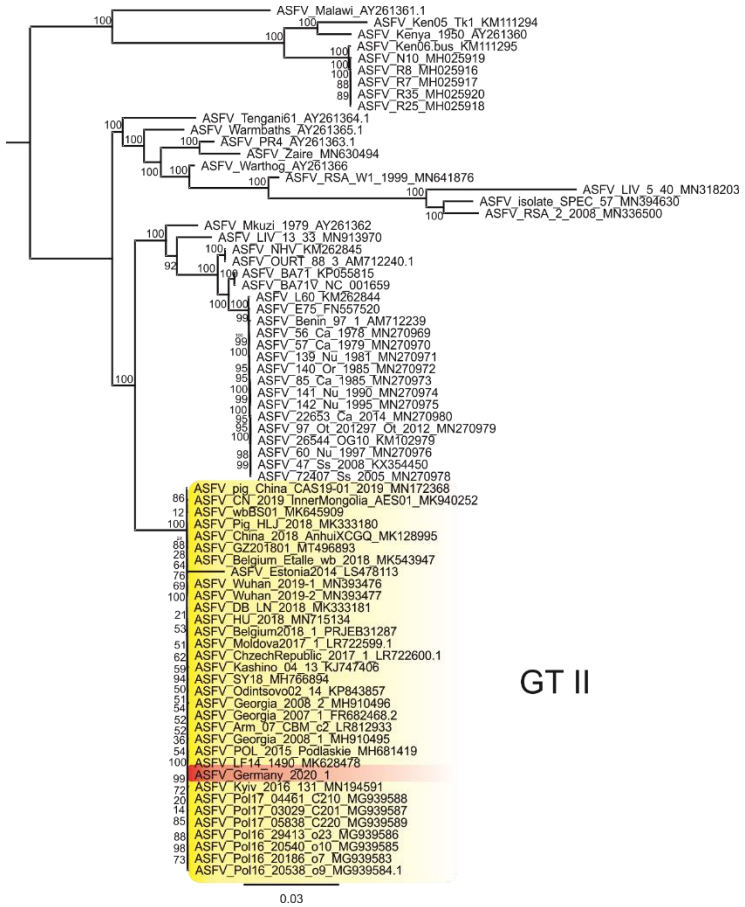
Alternative Sampling







ASFV Germany 2020



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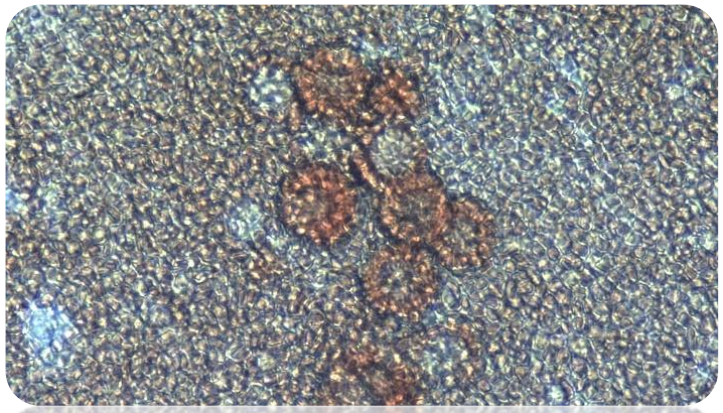
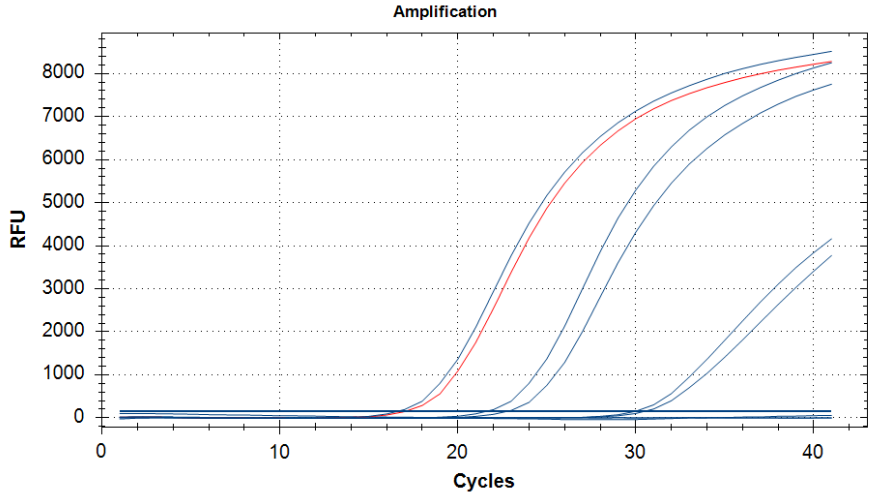


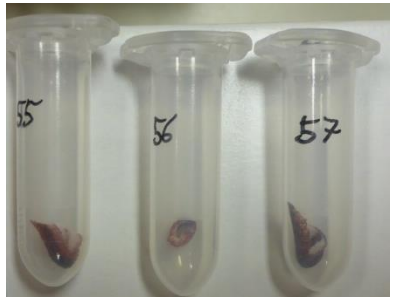
Some words about sample quality

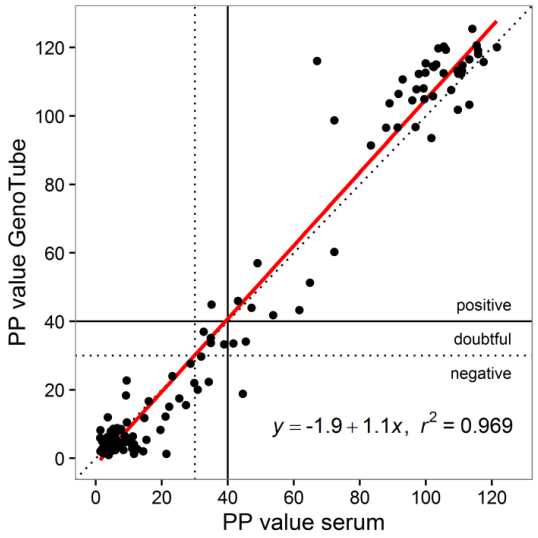
ID	County	Matrix	Age and sex	Sample origin	Stage of decomposition	LLBB IDEXX ASFV	NRL ASF King PCR	NRL ASF virotype ASFV	NRL ASF Tignon PCR
1	LK Spree-Neiße	Bone marrow	Adult, female	✞	III	24	23	21	25
2	LK Oder-Spree	Swab suspension	Subadult, female	●	I	30	29	27	29
3	LK Oder-Spree	Blood clot suspension	Piglet	✞	I	20	21	21	21
4	LK Oder-Spree	Swab suspension	Subadult, female	✞	II	30	25	29	25
5	LK Oder-Spree	Swab suspension	Adult, female	✞	II	25	25	24	25
6	LK Oder-Spree	Swab suspension	Piglet	✞	II	25	25	24	32
7	LK Oder-Spree	Swab suspension	Subadult, female	✞	II	24	26	23	nt
8	LK Oder-Spree	Swab suspension	Piglet	✞	II	28	28	25	29
9	LK Oder-Spree	Swab suspension	Piglet	●	I	26	25	24	27
10	LK Spree-Neiße	Bone marrow	Subadult, male	✞	III	27	30	27	31
11	LK Spree-Neiße	Bone marrow	Piglet	✞	III	33	35	29	37
12	LK Spree-Neiße	Bone marrow	Subadult, male	✞	III	29	*no cq*	33	38
13	LK Spree-Neiße	Bone marrow	Piglet	✞	III	31	36	30	37
14	LK Oder-Spree	Swab suspension	Subadult, female	✞	I	23	21	nt	nt
15	LK Oder-Spree	Bone marrow	Adult, female	✞	IV	30	33	31	34
16	LK Oder-Spree	Swab suspension	Piglet	✞	II	26	25	23	25
17	LK Spree-Neiße	Bone marrow	Piglet	✞	IV	37	35	*no cq*	34
18	**LK Spree-Neiße**	**Bone marrow**	**Piglet**	**✞**	**IV**	**19**	**20**	**17**	**20**
19	LK Spree-Neiße	Bone marrow	Piglet	✞	nk	21	18	17	19
20	LK Spree-Neiße	Bone marrow	Adult, female	✞	IV	23	23	22	24
21	LK Oder-Spree	Swab suspension	Piglet	✞	II	22	21	18	22
22	LK Oder-Spree	Swab suspension	Piglet	✞	II	26	25	24	27
23	LK Oder-Spree	Bone marrow	Female, no age differentiation	✞	IV	35	36	29	35
24	LK Oder-Spree	Bone marrow	Only bones, probably female	✞	IV	32	31	29	32
25	LK Oder-Spree	Bone marrow	Piglet	✞	IV	20	19	18	21
26	LK Oder-Spree	Bone marrow	Only bones, probably male	✞	IV	26	27	23	28
27	LK Oder-Spree	Bone marrow	Severily decoposed, no age and sex	✞	IV	28	27	26	29
28	LK Oder-Spree	Bone marrow	Adult, female	✞	III	26	25	24	27
29	LK Oder-Spree	Bone marrow	Severily decoposed, no age and sex	✞	IV	25	22	21	24
30	LK Oder-Spree	Bone marrow	Female, no age differentiation	✞	IV	20	24	21	nt
31	LK Oder-Spree	Bone marrow	Piglet	✞	II	19	19	18	nt
32	LK Oder-Spree	Swab suspension	Adult, male	✞	I	25	27	23	nt

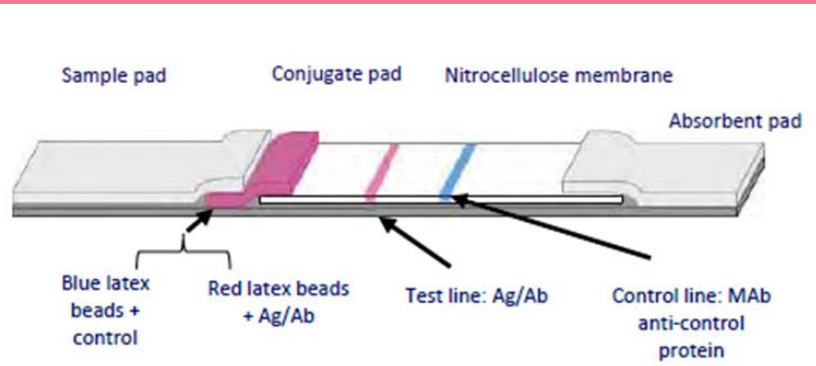


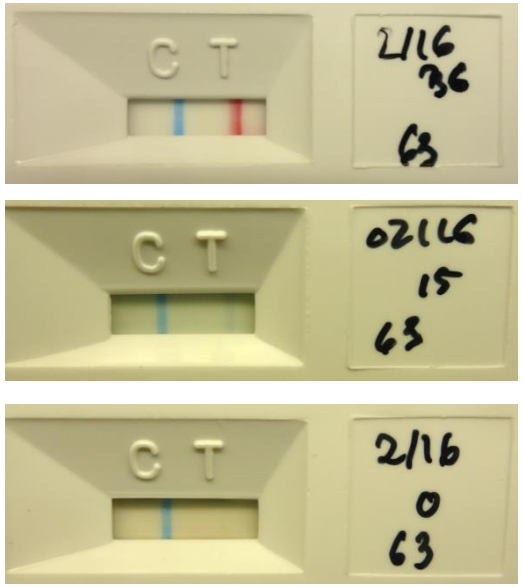
„Point-of-care“ Diagnostics

“Point-of-care„ Diagnostics

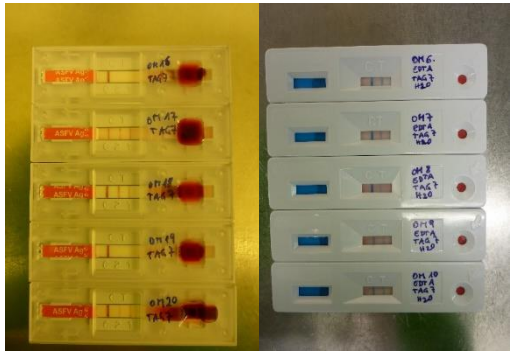








Can we use POCs in the field?



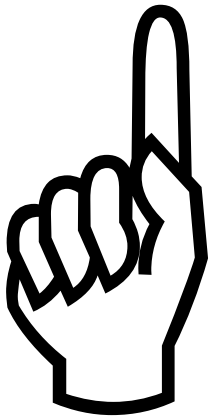
- Antibody LFDs are quite sensitive and highly specific
- Problem: Target animals may not have antibodies (passive surveillance)
- Antigen LFDs lack sensitivity but are also specific
- Sick pigs are usually detected under experimental conditions
- Data are needed to assess their suitability under field conditions
- Field-deployable genome detection methods are under development and evaluation; future will show their true value
- Who is going to test and report? What is the impact of positive/negative results!

About POC Sensitivity

About POC Sensitivity

Sample	DPI	Matrix	Pos./Neg.	PCR
TV 07/20 SUM	8	EDTA	positiv	ct 27
TV 07/20 SUM	8	EDTA	negativ	ct 30
TV 07/20 SUM	8	EDTA	positiv	ct 29
TV 07/20 KAB	8	EDTA	positiv	ct 27
TV 07/20 KAB	8	EDTA	negativ	ct 27
ASFV CHZT 90/01	8	EDTA	negativ	8,75E+04
ASFV CHZT 90/01	8	EDTA	positiv	6,16E+04
ASFV CHZT 90/01	8	EDTA	positiv	4,99E+04
ASFV CHZT 90/01	8	EDTA	positiv	8,45E+04
ASFV CHZT 90/01	8	EDTA	positiv	1,08E+05

- There is no general rule or value for “fitness for purpose„
- Diagnostic tests and their advantages and drawbacks have to be taken into account when designing the control and surveillance strategies



The bottom line:

Tests will only generate results

The challenge lies with sample choice and interpretation!

Serum 7	2020ASP00098	19,22	N/A	19,26	21,34	22,36	21,24	12,50	neg	88,50	neg
Serum 8	2020ASP00099	23,23	31,72	24,87	28,50	26,03	28,75	22,50	neg	87,50	neg
Serum 9	2020ASP00100	23,12	30,89	24,70	28,32	25,96	28,76	26,40	neg	83,40	neg
Serum 10	2020ASP00101	19,18	N/A	19,26	21,45	22,58	21,37	6,10	neg	81,90	neg
Serum 11	2020ASP00102	36,00	29,88	37,73	31,46	N/A	31,18	101,60	pos	-0,20	pos
Serum 12	2020ASP00103	21,09	N/A	20,65	21,93	24,53	21,56	57,20	pos	48,90	frag
Serum 13	2020ASP00104	20,66	35,28	20,62	21,85	23,61	21,54	257,20	pos	50,40	neg
Serum 14	2020ASP00105	N/A	29,13	37,34	31,12	N/A	30,78	102,90	pos	1,36	pos
Serum 15	2020ASP00106	21,46	31,51	22,13	23,46	24,40	24,05	40,00	frag	53,90	neg
Serum 16	2020ASP00107	21,35	32,34	22,08	23,47	24,12	23,83	41,70	frag	54,30	neg

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GARA
Global African Swine Fever Research Alliance

Thanks for your attention!