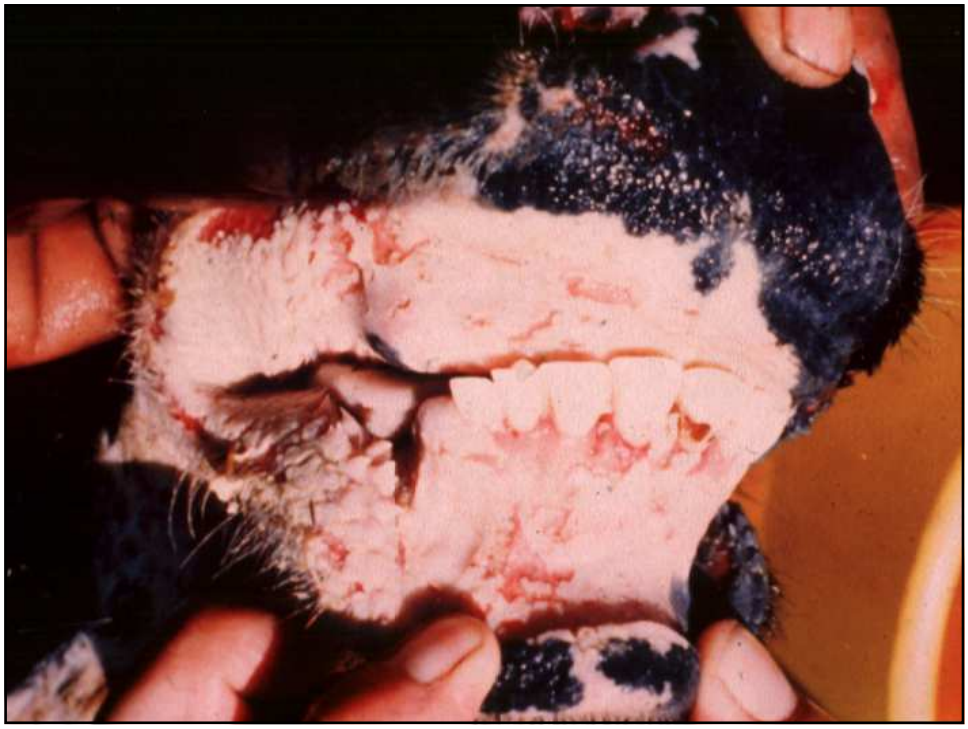
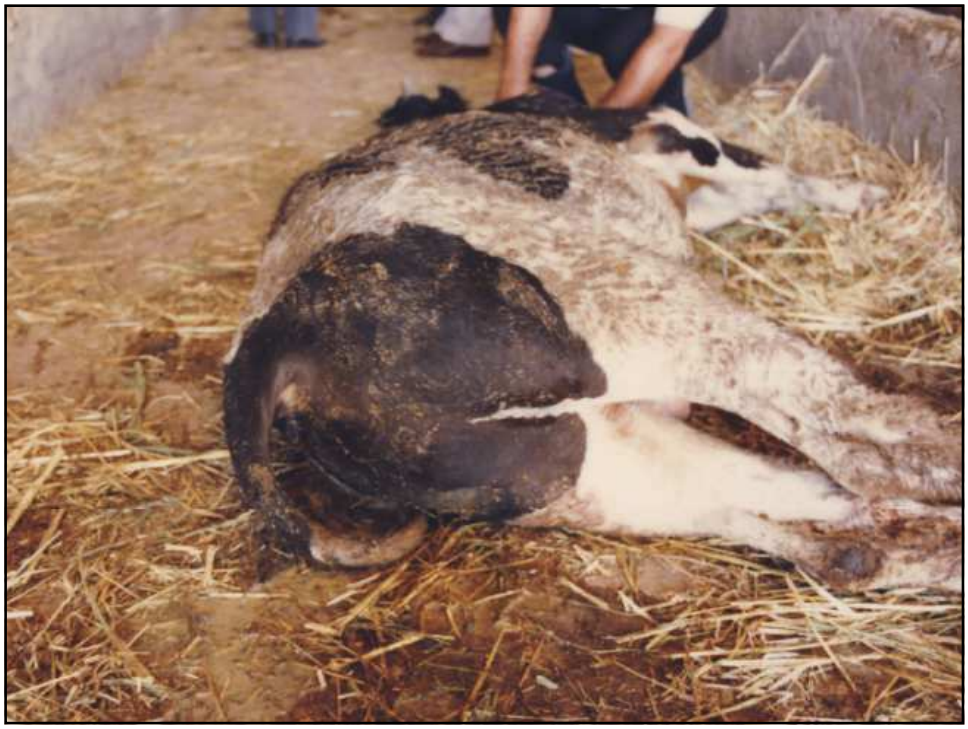
August 8, 1990

Do you remember where you were?

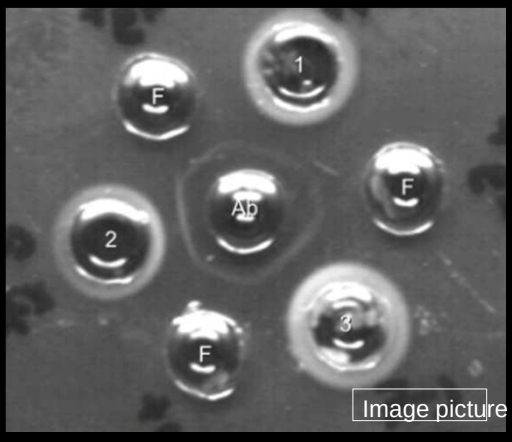
I was in Baghdad, IRAQ

Facing an outbreak of a disease…





Disease was diagnosed as Rinderpest by Agar Gel Immuno-diffusion Test (AGID)



Point-of-Need Test in 1990

25 years later…
We have…

Point-of-Need PCR
(PONP, Pen-side PCR)

Evaluation and application
for
H7N9 and ASF

Ken Inui and Filip Claes
ECTAD, FAO

Application of Point-of-Need PCR (Pen-side PCR) for H7N9 surveillance in Vietnam

1. Increased risk of H7N9 introduction to VN (winter 2016-2017)
2. Pilot study on the field use of Pen-side PCR in 5 provinces (March – May 2017)
3. Validation of Pen-side PCR for H7N9 detection in collaboration with HKU (October 2017)
4. Rolling out of H7N9 surveillance using Pen-side PCR in 5 provinces (Jan – April 2018)

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H7N9 Risk Assessment Meeting at MoH

- MoH
- MoA
- WHO
- FAO
- CDC

13 of 16 people said Yes to Q "H7N9 introduction to Vietnam???"

H7N9 risk assessment at MoH

2019/6/24

What is Point-of-Need PCR (Pen-side PCR)?

- Pen-side PCR is a portable PCR system that can be used for pathogen detection at/near the sampling sites such as farm, market, slaughter house, veterinary stations, etc.
- "Pockit iiPCR system" developed by GeneReach (China) was selected for H7N9 surveillance as it provides the total solution (from equipment to reagent) that includes
  - Portable equipment for RNA extraction and PCR
  - Easy to use reagent kits (lyophilized thermo-stable PCR reagent)
  - Simple test procedure and result interpretation
  - Commercially available at affordable cost
  - Validated for various pathogens (FMD, CSF, etc.)

Automatic RNA extraction
Taco mini $5,000-
Reagent: $3-/sample

PCR device
Pockit plus $2,000-
Reagent:
$8-/reaction

GeneReach Pockit iiPCR System

Various choice/combination of **tools** for Various settings/**needs**

Settings/needs	DNA/RNA extraction	Probe-based iiPCR
Field • Farm • Local vet service • Vet medicine supplier	 Grind-N-Go (no equipment needed)	  Pockit micro (4 wells) (on battery) Pockit (8 wells)
Field / Small lab • Farm • Live bird market • Slaughter house • Local vet service • Vet medicine supplier	  Taco mini (8) (on battery)	
Small Lab / etc • Large farm • Local vet service • Vet medicine supplier	 Pockit central (8 wells)	

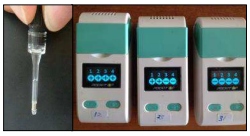
Why Pen-side PCR for H7N9 surveillance?

- H7N9 virus emerged in China 2013, has infected more than 1600 humans till now (Mar 2018). Vietnam share the long border with China and is at high risk of H7N9 introduction
- Challenge of the current H7N9 surveillance is the time to get lab test results after sampling = 2.5 days. Most time spent for sample transportation.
- Quick detection of H7N9 virus in the surveillance is essential for quick response and containment of virus
- Use of Pen-side PCR at/near sampling site will enable to get test results in 2 to 3 hours after sampling (0 - 1 hr for sample transportation, 2.0 hr for testing)

Test immediately after sampling

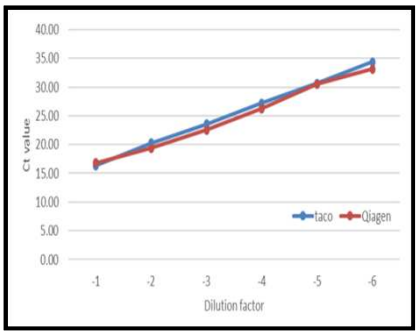
Testing procedure of Pen-side PCR (Taco Mini and Pockit)

RNA extraction by Taco Mini	iiPCR by Pockit
1. Open cover of pre-loaded plate 2. Add 200ul of sample to the first row 3. Set plate to Taco Mini 4. Run 5. Wait for 25 minutes 6. Collect 100ul of RNA solution from the last row to 2.0ml microcentrifuge tube	1. Add 50ul of reaction buffer to iiPCR reagent tube 2. Add 5ul of RNA solution to iiPCR reagent tube 3. Transfer 50ul to iiPCR reaction tube (R-tube) 4. Hand-centrifuge R-tube 5. Set iiPCR reaction tubes to Pockit and run 6. Wait for 40 minutes 7. Read results (positive / negative)

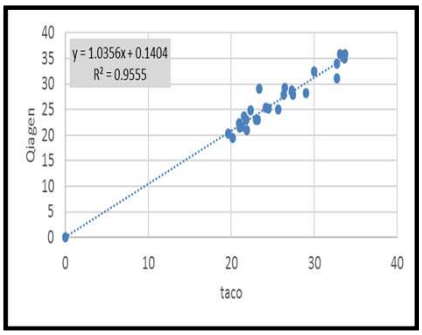


Efficiency of RNA extraction by Taco Kit compared with Qiagen RNeasy Mini Kit

- Results of test 1 and 2 showed that both kits have equivalent level of efficiency in extracting RNA from samples



Test 1: Samples are H7N9 virus serially diluted 10-fold



Test 2: Samples are OP swabs collected in the field

Validation of Pockit iiPCR (Pen-side PCR) for the detection of H7N9 virus (in comparison with Lab-based qPCR)

@ HKU joint Influenza Research Center, Shantou Medical University in Shantou, Guangdong, China

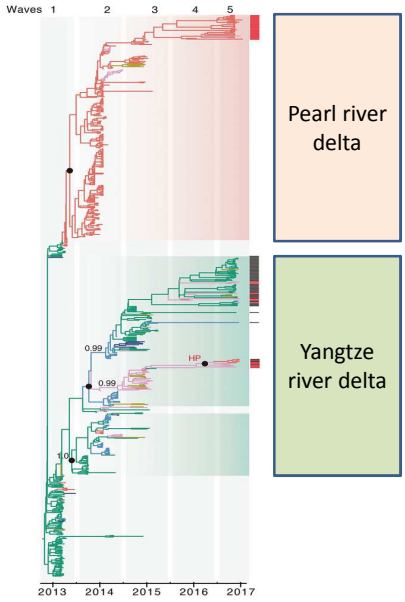
Objectives

Validate Pockit iiPCR for the detection of H7and N9 genes in comparison with Lab-based qPCR by examining the followings

- Analytical sensitivity and specificity (isolated virus)
- Diagnostic sensitivity and specificity (clinical samples)

Genetic evolution and spatial spread of epidemic lineage of influenza A(H7N9) viruses, China, 2013–2017

Source
Lu J, Raghwani J, Pryce R, Bowden TA, Thézé J, Huang S, et al. Molecular Evolution, Diversity, and Adaptation of Influenza A(H7N9) Viruses in China. Emerg Infect Dis. 2018;24(10):1795-1805. https://dx.doi.org/10.3201/eid2410.171063



Materials and Methods (1/2)

Viruses used for the study

Objectives		Materials			Methods	
		Viruses (number)	Virus details (number)	RNA	Lab-based qPCR	Pockit iiPCR
Analytical	Sensitivity	H7N9 virus (4)	2013 Anhui (1)	Extracted RNA were diluted 10-fold up to 10(-8)	Tested with 3 primer sets.	Tested with 4 primer sets.
			2017 Pearl lineage (1)			
			2017 Yanzee lineage (1)			
			2017 Highly pathogenic (1)			
	Specificity	H7N9 virus (24)	2017 Pearl lineage (10)	RNA were extracted from isolated viruses		
			2017 Yanzee lineage (10)			
			2017 Highly pathogenic (4)			
		Other Flu A virus (30)	H7 viruses not H7N9 (7)			
			Avian virus of subtype H1-H13 (15)			
			swine virus (3)			
			human virus (4)			
		Poultry virus (2)	NDV (1), IBD (1)			
Diagnostic	Sensitivity	H7N9 positive swabs (50)	chicken & duck swabs (25 each) from experimental infection	RNAs were extracted by 2 methods (Taco, QiaAmp)	RNA extracted by QiaAmp and tested by 1 primer set	RNA extracted by Taco and tested by 2 primer sets
	Specificity	H7N9 negative swabs (50)	chicken & duck swabs (25 each) from experimental infection			

Materials and methods (2/2)

Primer set used for comparison

Subtype	Target	Purpose	Primer set used	
			Lab qPCR	Pockit iiPCR
H7	H7 Wide	Detect most H7 viruses Eurasian lineage	CODA	GR
	H7 CN	Detect Chinese H7N9 more specifically	CNIC/WHO	CNIC/WHO
	H7 HP	Detect HPAI H7 but not LPAI H7 (differentiate HPAI from LPAI)	HVRI	HVRI
N9	N9	Detect N9 gene (it is not specific to H7N9)		CNIC/WHO

CODA: CODA-CERVA (Veterinary and Agrochemical Research Institute) in Belgium

GR: GeneReach (Company who developed Pockit iiPCR)

CNIC: China National Influenza Center

HVRI: Harbin Veterinary Research Institute

Result (1/3)

Analytical sensitivity

Virus	Limit of Detection (the highest dilution that gave positive in all tripricates)						
	Lab-based qPCR			Pockit iiPCR			
	H7 Wide	H7 CN	H7 HP	H7 Wide	H7 CN	H7 HP	N9
	CODA	CNIC	HVRI	GR	CNIC	HVRI	CNIC
2013 Index virus	-5	-5	neg	-6	-4	neg	-4
2017 Pearl lineage	-6	-4	neg	-6	-4	neg	-4
2017 Yanzee lineage	-6	-5	neg	-6	-5	neg	-5
2017 HPAI H7	-6	-4	-5	-6	-5	-6	-5

LOD (limit of detection) determined by manufacturer using in-vitro transcribed RNA of Pockit iiPCR is 11 copies for H7GR, 16 copies for H7CNIC, 261 copies for H7HVRI, 278 copies for N9CNIC

Result (2/3)
Analytical specificity

Virus	Detection rate % (No of positive / No of tested)						
	Lab-based qPCR			Pockit iiPCR			
	H7 Wide	H7 CN	H7 HP	H7 Wide	H7 CN	H7 HP	N9
	CODA	CNIC	HVRI	GR	CNIC	HVRI	CNIC
2017 Pearl lineage	100%	100%	70%	100%	100%	80%	100%
2017 Yanzee lineage	100%	100%	30%	100%	100%	70%	100%
2017 HPAI H7	100%	100%	100%	100%	100%	100%	100%
H7 virus not CNH7N9	100%	57%	0%	100%	86%	29%	0%
AI of other subtype	0%	0%	0%	0%	0%	0%	0%
Swine Flu virus	0%	0%	0%	0%	0%	0%	0%
Human Flu virus	0%	0%	0%	0%	0%	0%	0%
Poultry virus	0%	0%	0%	0%	0%	0%	0%

Result (3/3)
Diagnostic sensitivity and specificity

- Virus isolation vs Pockit iiPCR
  - Sensitivity= 49/(49+1) = 98%
  - Specificity= 50/(50+0) = 100%
  - Kappa = 0.98, p < 0.001

		Pockit iiPCR H7GR		Total
		Positive	Negative	
Virus isolation	Positive	49	1	50
	Negative	0	50	50
Total		49	51	100

- Lab qPCR vs Pockit iiPCR
  - Sensitivity= 49/(49+1) = 98%
  - Specificity= 38/(38+0) = 100%
  - Kappa = 0.98, p < 0.001

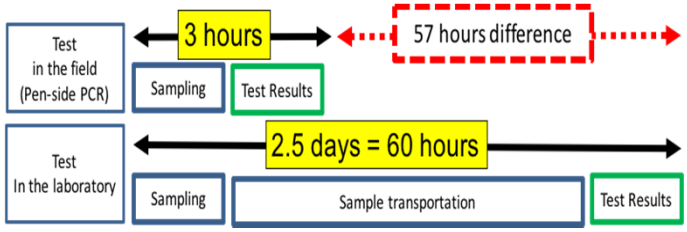
		Pockit iiPCR H7GR		Total
		Positive	Negative	
Lab qPCR H7CODA	Positive	49	1	50
	Negative	0	38	38
Total		49	39	88

Results

- Pockit iiPCR (primer H7GR) had equivalent level of analytical sensitivity and specificity with Lab-based qPCR (primer H7CODA) for the detection of H7 gene of Eurasian lineage
- Diagnostic sensitivity and specificity of Pockit iiPCR (H7GR) are 98% and 100% respectively compared with Lab-based qPCR and virus isolation
- Pockit iiPCR with primer N9CNIC had one log lower sensitivity compared with Pockit H7GR and 100% specificity for the detection of N9 gene

Discussions and Conclusions

- The validation study showed that Pockit iiPCR would provide the same level of sensitivity and specificity compared with Lab-based qPCR for the detection of H7 (and N9 genes).
- Early detection of H7N9 virus by the use of Pen-side PCR (Pockit iiPCR) near the sampling sites followed by quick response will reduce the risk of virus infection and spread in humans and poultry.



Influenza and Other Respiratory Viruses. 2019;00:1–8.

ORIGINAL ARTICLE

WILEY

A field-deployable insulated isothermal RT-PCR assay for identification of influenza A (H7N9) shows good performance in the laboratory

Ken Inui[1] | Nguyen Tung[2] | Hsin-Jou Tseng[3] | ChuanFu Mark Tsai[3] | Yun-Long Tsai[3] | Simon Chung[3] | Pawin Padungtod[1] | Huachen Zhu[4,5,6] | Yi Guan[4,5,6] | Wantanee Kalpravidh[7] | Filip Claes[7]

[1]Food and Agriculture Organization of the United Nations (FAO), Hanoi, Vietnam
[2]Department of Animal Health, Hanoi, Vietnam
[3]GeneReach USA, Lexington, Massachusetts
[4]Joint Institute of Virology (Shantou University – The University of Hong Kong), Shantou, China
[5]State Key Laboratory of Emerging Infectious Diseases, School of Public Health, The University of Hong Kong, Hong Kong, China
[6]Shenzhen Third People's Hospital, Shenzhen, China
[7]Food and Agriculture Organization of the United Nations (FAO), Regional office for Asia and the Pacific, Bangkok, Thailand

When we use Pen-side PCR…

Birds are still there when results are up for response

Pen-side PCR: Requirements

1. Performance = validation
2. Biosafety
3. Usability by non-specialist users
4. Portability
5. Speed
6. Cost and availablity
7. Wide application for other livestock diseases

Requirement 2: Biosafety

Samples are collected into 2 kinds of medium

A: Lysis buffer

This will be used for Pen-side PCR testing. Live pathogen are inactivated in this buffer removing biosafety concerns during testing in the field.

B: Virus transport medium

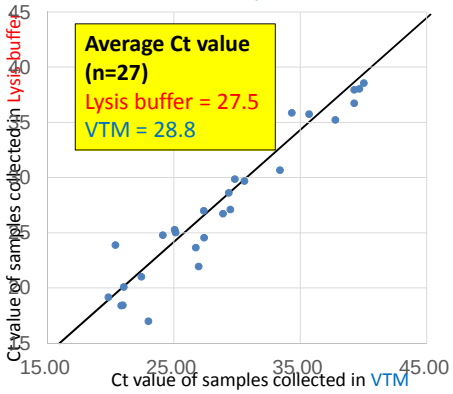
The samples collected in this medium will be kept at 4C in case further testing/confirmation are required in the laboratory.

2019/6/24

Comparison of 2 kinds of sample collection medium on the efficacy of viral RNA recovery

Lysis buffer (sample storage buffer) vs VTM (virus transport medium)

- 2 OP swabs were collected from one bird. One swab stored in lysis buffer, and the other in VTM. RNA extracted from both were tested for FluA by qPCR at the same time.
- Average Ct value of samples
  - 27.5 for lysis buffer
  - 28.8 for VTM
- Recovery of RNA is x2 more in lysis buffer than VTM



Requirement 3: Usability by non-specialist users

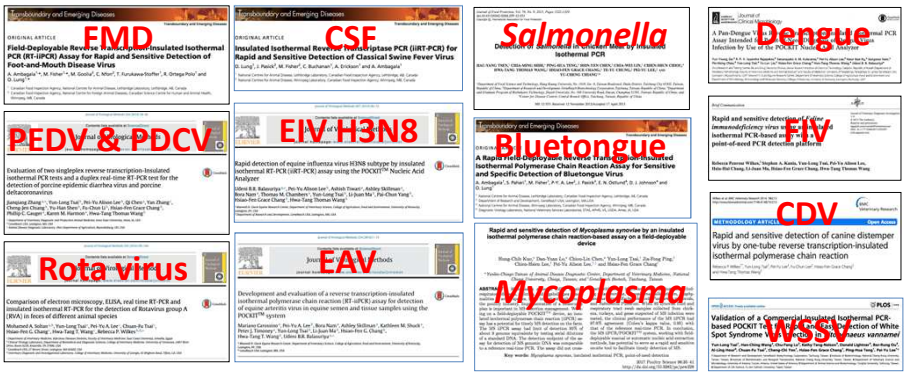
Pilot study in 4 provinces in Vietnam

Requirement 4, 5: Portability & Speed

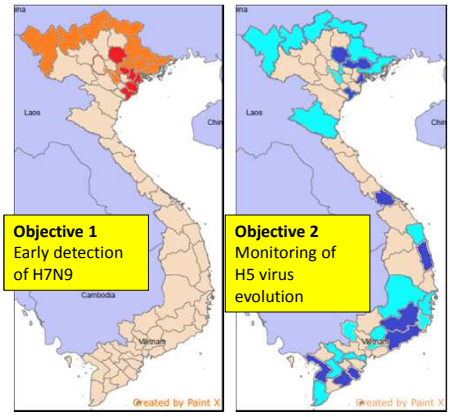
- **PCR (Pockit): 0.38kg, Hand-held**
  - Battery-operated; 5 runs per charge; charge by USB connection (Pockit)
  - Reagent: Lyophilized; can be kept at room temperature
  - Run time: 40 minutes
- **RNA extraction (Taco mini): 5.5kg**
  - Battery-operated; 6 runs per charge
  - Reagent: pre-loaded; ready-to-run; store at room temperature
  - Run time: 25 minutes

Requirement 7: Wide applications for other pathogens

Validated for various Pathogens in livestock, aquaculture, pets, humans = worth investing on equipment



Avian Influenza Surveillance 2018 in Viet Nam



Routine surveillance

Intensive H7N9 surveillance using Pen-side PCR

- 5 months (Dec – April)/year
- 5 provinces in the north
- Total 11 LBMs (in 5 provinces)
- Sample 2 times/week
  - 40x11x2x4=3520 samples/month

Additional H7N9 surveillance

Thanks to

- USAID
- Hong Kong Univ
- Sub-department of animal health in Quang Ninh, Lang Son, Cao Bang, Lao Cai
- Animal quarantine office in Quang Ninh, Lang Son, Lao Cai
- All the colleagues in the field

African Swine Fever

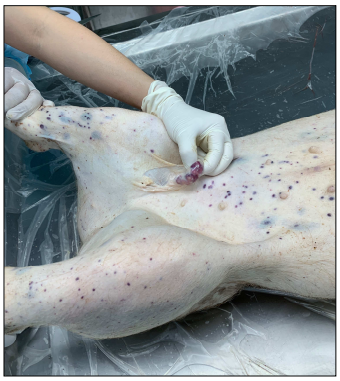
Lessons learned in Viet Nam

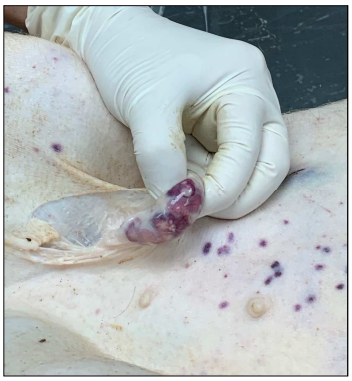
ASF virus tissue tropism

No.	Organs	Level of ASF viral DNA in organ by Ct value							
		Pig No.1 (7wks old, dead)				Pig No.2 (7wks old, sick and killed)			
		1	2	3	Mean	1	2	3	Mean
1	**Heart**	20.65	20.38	20.82	**20.6**	19.89	20.45	20.51	**20.3**
2	**Lung**	17.59	18.01	17.89	**17.8**	16.52	16.83	16.24	**16.5**
3	**Liver**	15.93	15.83	15.22	**15.7**	17.10	16.55	16.29	**16.6**
4	**Kidney**	20.82	21.23	21.19	**21.1**	19.83	20.02	20.98	**20.3**
5	**Muscle**	24.22	23.62	23.91	**23.9**	23.73	23.09	23.20	**23.3**
6	**Tonsil**	15.39	15.82	15.69	**15.6**	18.78	19.41	19.38	**19.2**
7	**Spleen**	13.98	14.48	14.72	**14.4**	15.95	15.62	15.33	**15.6**
8	**Lymph node (mesenteric)**	16.55	17.04	16.99	**16.9**	18.19	19.24	18.73	**18.7**
9	**Lymph node (mandibular)**	17.30	17.29	16.89	**17.2**	18.50	18.21	18.72	**18.5**
10	**Lymph node (inguinal)**	16.92	16.60	17.12	**16.9**	18.59	18.64	18.70	**18.6**
11	**Blood (serum)**	NA	NA	NA	**NA**	18.50	19.23	18.79	**18.8**

Sample collection for lab diagnosis of ASF suspected case

- Requirement
  - Easy to collect
  - No to open animals = Less virus contamination of the environment
- Collection of **inguinal lymph node** (figure) from 3 dead pigs





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GeneReach Pockit iiPCR System

Various choice/combination of **tools** for Various settings/**needs**

Settings/needs	DNA/RNA extraction	Probe-based iiPCR
Field • Farm • Local vet service • Vet medicine supplier	 Grind-N-Go (no equipment needed)	
Field / Small lab • Farm • Live bird market • Slaughter house • Local vet service • Vet medicine supplier	  Taco mini (8) (on battery)	  Pockit micro (4 wells) (on battery) Pockit (8 wells)
Small Lab / etc • Large farm • Local vet service • Vet medicine supplier	 Pockit central (8 wells)	

Validation of Pen-side PCR using ASF virus genotype 2 in Viet Nam (taco Mini + Pockit micro plus)

- Analytical sensitivity and specificity
  - Sensitivity one log higher than real-time PCR
  - Negative for FMD, CSF, PRRS, PED, TGE
- Diagnostic sensitivity and specificity
  - 100% sensitivity
  - 100% specificity

Table 1. Analytical sensitivity in comparison with real-time PCR

Viral DNA dilution	Test results							
	Real-time PCR (Ct value)				POCKIT iiPCR			
	1	2	3	% positive	1	2	3	% positive
-4	31.2	30.9	31.4	100	Pos	Pos	Pos	100
-5	35.4	35.2	35.2	100	Pos	Pos	Pos	100
-6	neg	38.5	38.3	67	Pos	Pos	Pos	100
-7	neg	neg	neg	0	neg	neg	neg	0

Table 2. Diagnostic sensitivity and specificity using clinical samples

		POCKIT iiPCR			Sensitivity	Specificity
		Positive	Negative	Total		
Real-time PCR	Positive	20	0	20	100%	
	Negative	0	20	20		100%
	Total	20	20	40		

GeneReach Pockit iiPCR System

Various choice/combination of **tools** for Various settings/**needs**

Settings/needs	DNA/RNA extraction	Probe-based iiPCR
Field • Farm • Local vet service • Vet medicine supplier	Drop-N-Go, Grind-N-Go (no equipment needed)	
Field / Small lab • Farm • Live bird market • Slaughter house • Local vet service • Vet medicine supplier	Taco mini (8) (on battery)	Pockit micro (4 wells) (on battery) Pockit (8 wells)
Small Lab / etc • Large farm • Local vet service • Vet medicine supplier	Pockit central (8 wells)	

Validation of Pen-side PCR using ASF virus genotype 2 in Viet Nam (Pockit Central)

- Analytical sensitivity and specificity
  - Sensitivity one log higher than real-time PCR
  - Negative for FMD, CSF, PRRS, PED, TGE
- Diagnostic sensitivity and specificity
  - 100% sensitivity
  - 100% specificity

Table 1. Analytical sensitivity in comparison with real-time PCR

Sample dilution	Test results							
	Real-time PCR (Ct value)				POCKIT iiPCR			
	1	2	3	% positive	1	2	3	% positive
-4	28.48	28.79	28.95	100	Pos	Pos	Pos	100
-5	34.68	32.41	32.33	100	Pos	Pos	Pos	100
-6	neg	neg	neg	0	Pos	Pos	Pos	100
-7	neg	neg	neg	0	neg	neg	neg	0

Table 2. Diagnostic sensitivity and specificity using clinical samples (serum)

		POCKIT iiPCR			Sensitivity	Specificity
		Positive	Negative	Total		
Real-time PCR	Positive	31	0	31	100%	
	Negative	0	29	29		100%
	Total	31	29	60		

Potential application of portable PCR for ASF

- Diagnosis of suspected cases
  - in remote area (far away from laboratory)
  - 2ndary cases in the affected zone
- Pig movement control points
  - Check points in/out affected zones or between provinces
  - Quarantine stations
- Farms
  - Routine testing of mortality
  - Checking for virus contamination of incoming materials such as feed, semen, equipment, etc.
  - Checking ASF infection of replacement pigs
- Slaughter houses
  - Monitoring of ASF contamination of incoming pigs
  - Monitoring of raw meat

CLINICAL SIGNS OF ASF

A case study: Slow spread within a herd

Date	Sow (n=21)					Weaner (n=23); Piglets (n=49)
	No. 1 (Gilt)	No. 2 (Gilt)	No. 3 (Sow)	No. 4, 5 (Sow)	No. 6, 7, 8 (Sow/Gilt)	Pig of 1-2 months old (n=23)
Day 1	Sick					
Day 4	DEAD					
Day 5		Sick				
Day 9		Culling				
Day 12			Sick			
Day 16			DEAD			
Day 20				Sick		
Day 22						Piglets still eat but reduce
Day 24				DEAD/ KILLED		
Day 25						Piglets almost give up eating 3 piglets DIED
Day 26					Sick	5-7 piglets DIED
Day 27					Culled	5-7 piglets DIED/day
Day 34	Remaining 13. sows					All piglets were eliminated by owner, excep for 4 weaned piglets and 10 piglets followed mother were kept. These pigs were
Day 35 (February 1, 2019)	Sera samples were sent to Dr. Phan' lab and diagnosed as ASF- positive. Outbreak was reported to DAH and all pigs were stamping-out by government for the same day					

Information kindly shared by Prof. Le Van Phan, Vietnam National Agriculture University

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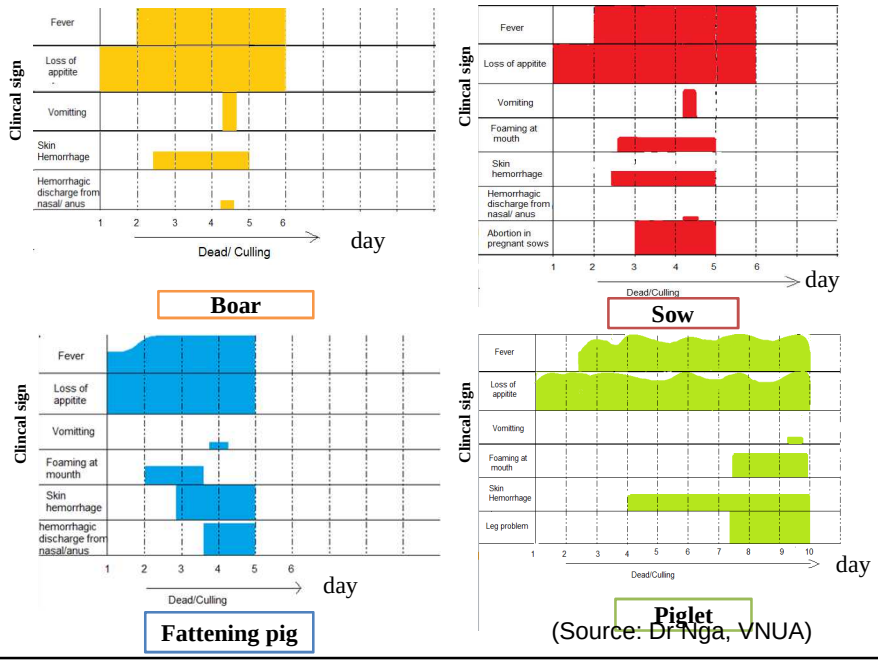
Clinical signs

Clincal signs	Type of pig			
	Boar (%) (n=3)	Sow (%) (n=178)	Fattening (%) (n=212)	Piglet (%) (n=93)
Fever	**100**	**100**	**100**	**100**
Loss of appitite	**100**	**100**	**100**	**100**
Vomiting	**100**	**90**	10	20
Foaming at mouth	0	40	55	**80**
Skin hemorrhage	33	40	**100**	50
Hemorrhagic discharge from nasal/anus	10	10	**90**	0
Abortion in pregnant sows	-	**100**	-	-
Leg problem	0	0	0	**100**

(Source: Dr Nga, VNUA)

Disease course



(Source: Dr Nga, VNUA)