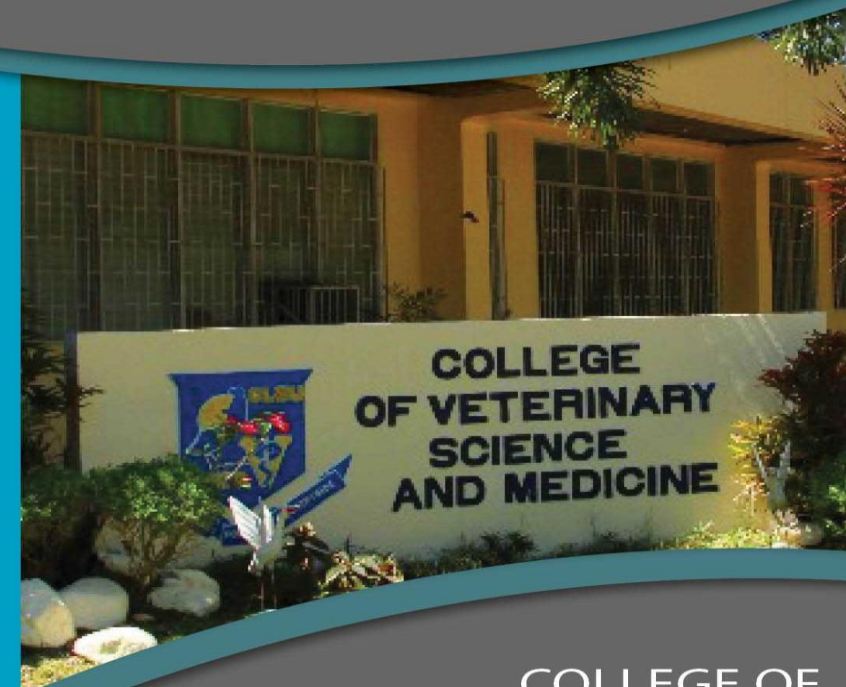


TITLE OF PAPER

RT-LAMP Assay for the Detection of
Classical Swine Fever (CSF) and Porcine
Reproductive and Respiratory Syndrome
(PRRS) Vaccine Virus Shedding



COLLEGE OF
**VETERINARY
SCIENCE AND
MEDICINE**

"dedicated to service and excellence"



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IMPLEMENTING AGENCY/STATION

College of Veterinary Science & Medicine,
Central Luzon State University



COLLEGE
OF VETERINARY
SCIENCE
AND MEDICINE



FUNDING AGENCY: DOST-PCAARRD

PROJECT DURATION

Date Started: January 2016

Expected Date of Completion: December 2018



OBJECTIVES

General Objective

To formulate a dry format RT-LAMP protocols for CSFV and PRRS that could differentiate infected from vaccinated animals.

Specific Objectives

1. To characterize the molecular phylogenecity of the vaccine and the field strain viruses of CSF and PRRS coming from backyard and commercial swine farms in Luzon.
2. To formulate a dry format RT-LAMP and RT-RPA for both viruses.



EXPECTED OUTPUTS AND DERIVATIONS

Information/knowledge on the genetic strain of CSF and PRRS virus field strain and the viruses used in the current vaccination of CSF and PRRS.

1. Developed the CSFV and PRRSV RT-LAMP dry format protocols
2. Validated CSFV and PRRSV RT-LAMP protocols
3. Assembled CSFV and PRRSV RT-LAMP test kits for easy handling
4. Submitted to IPO for patent of DIVA test kits for CSF and PRRS detection

Background of the study

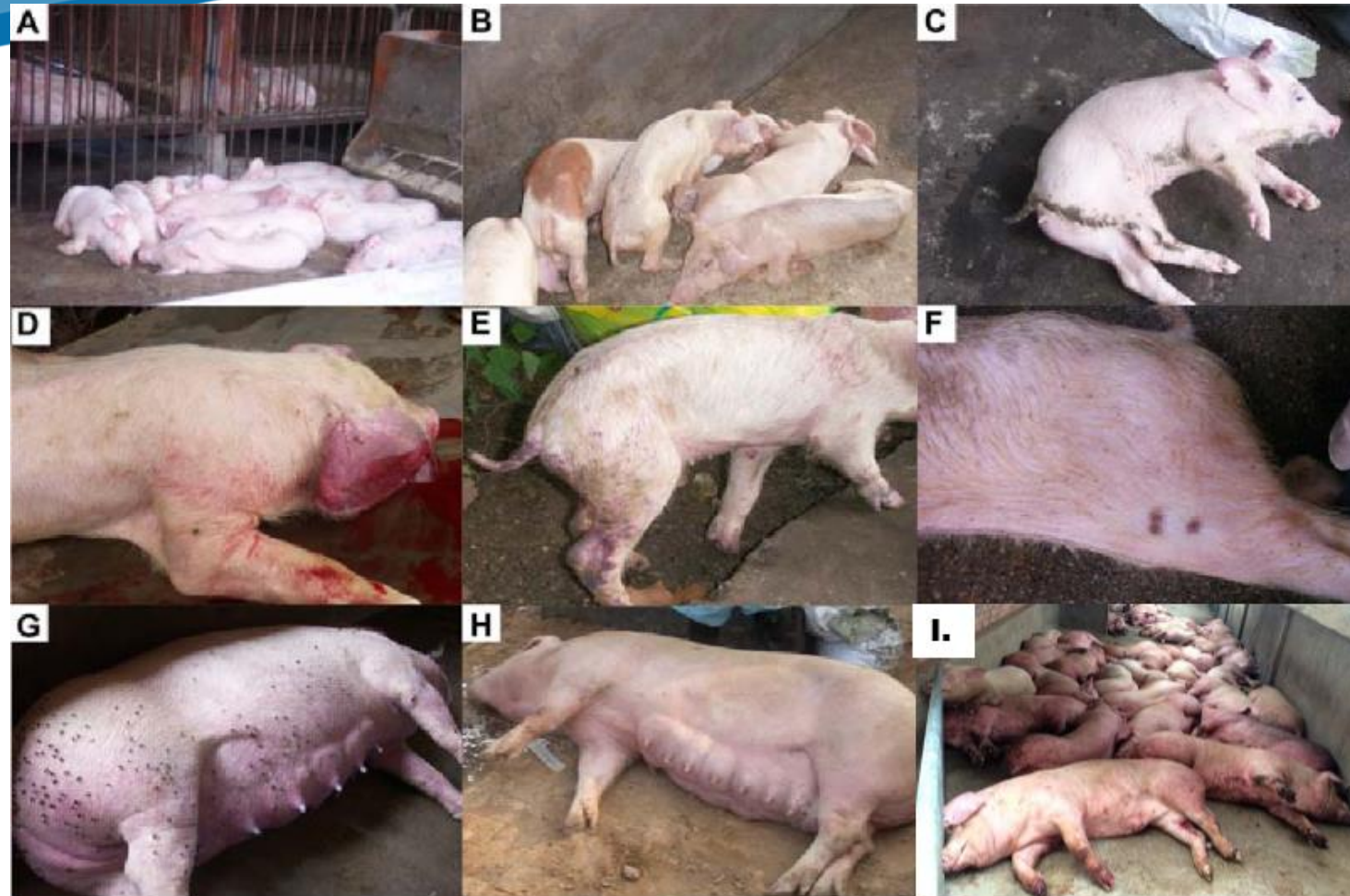


Fig.1 Clinical presentation of pigs with “high fever” disease. (A) Sick pigs with a fat and healthy appearance. **(B)** Sick pigs with thin and debilitated features. **(C)** The shivering piglet. **(D)** The limping pig with erythematous blanching rash in its ears. **(E)** & **(F)** Pimples observed on the back of an infected pig. **(G)** & **(H)** Grown pigs killed during this epidemic. doi:10.1371/journal.pone.0000526.g002 *China, 2007*

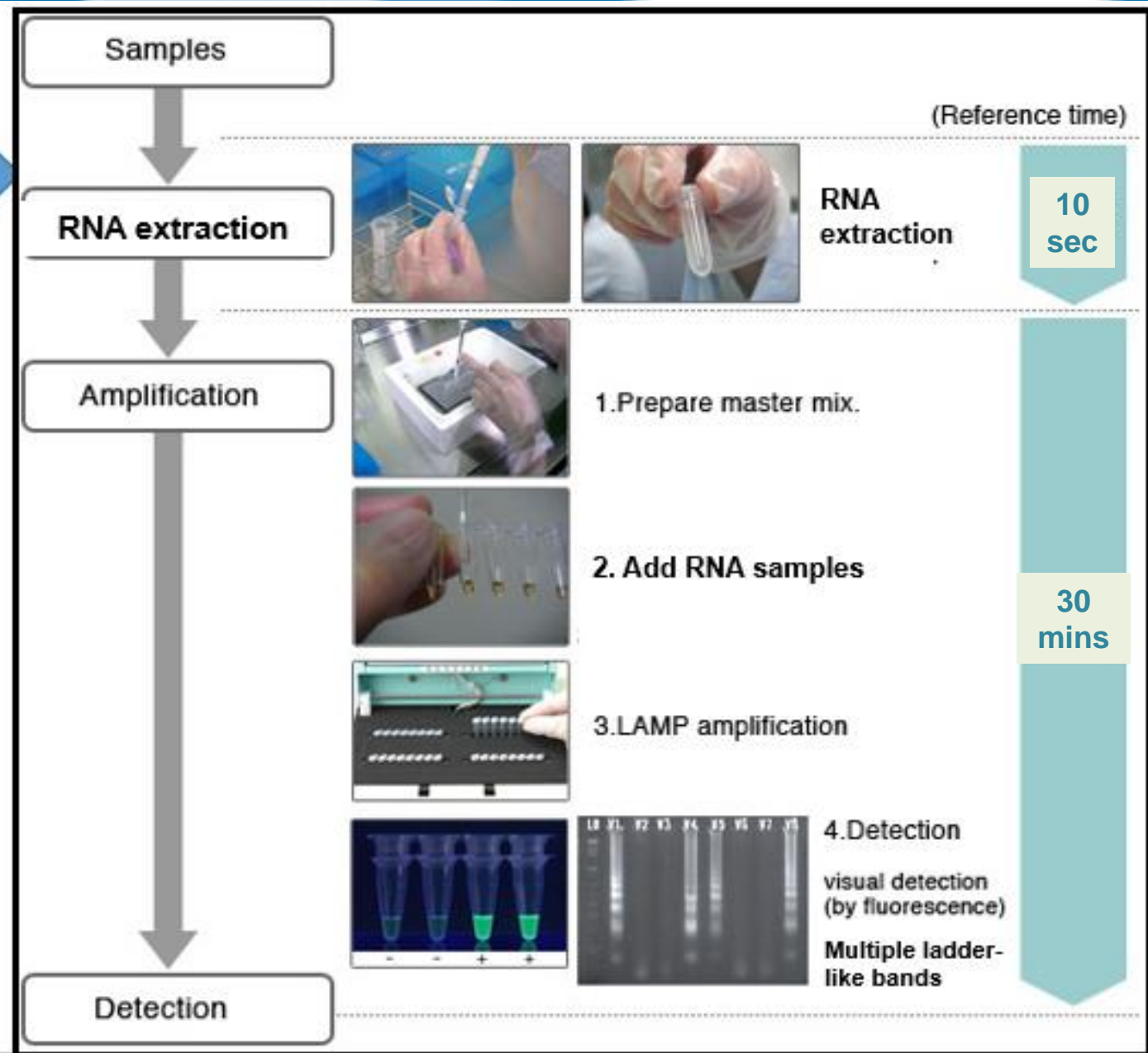
WHO ASSURED GUIDELINES FOR AN IDEAL DIAGNOSTIC TEST

A	ffordable
S	ensitive
S	pecific
U	ser-friendly
R	obust and rapid
E	quipment-free
D	eliverable to the end user

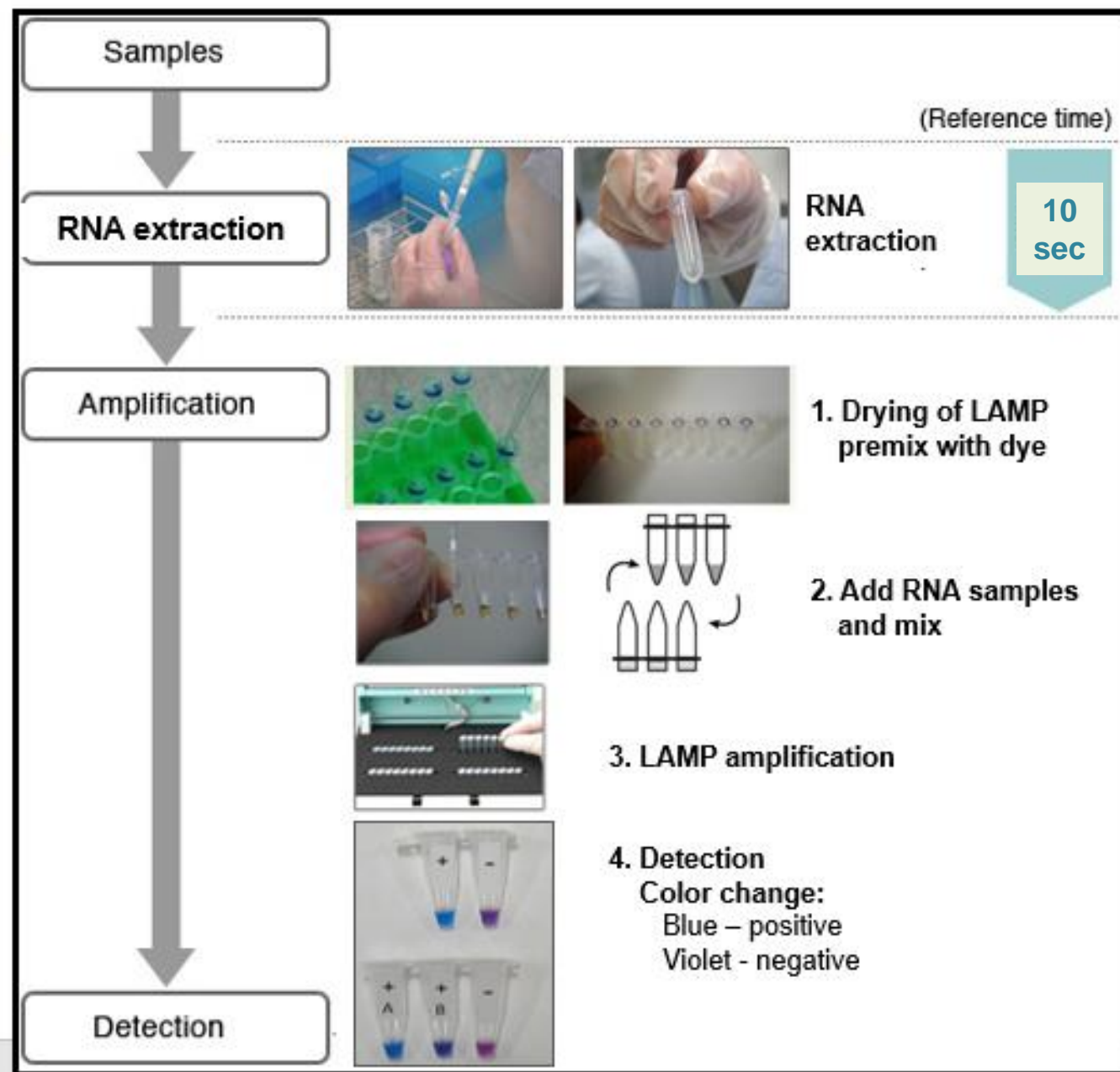
RT-PCR	RT-LAMP
X	/
/	/
/	/
/	/
X	/
X	/ ?
/	/

RT-RPA
/
/
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/
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RT-LAMP Wet Format



RT-LAMP Dry Format



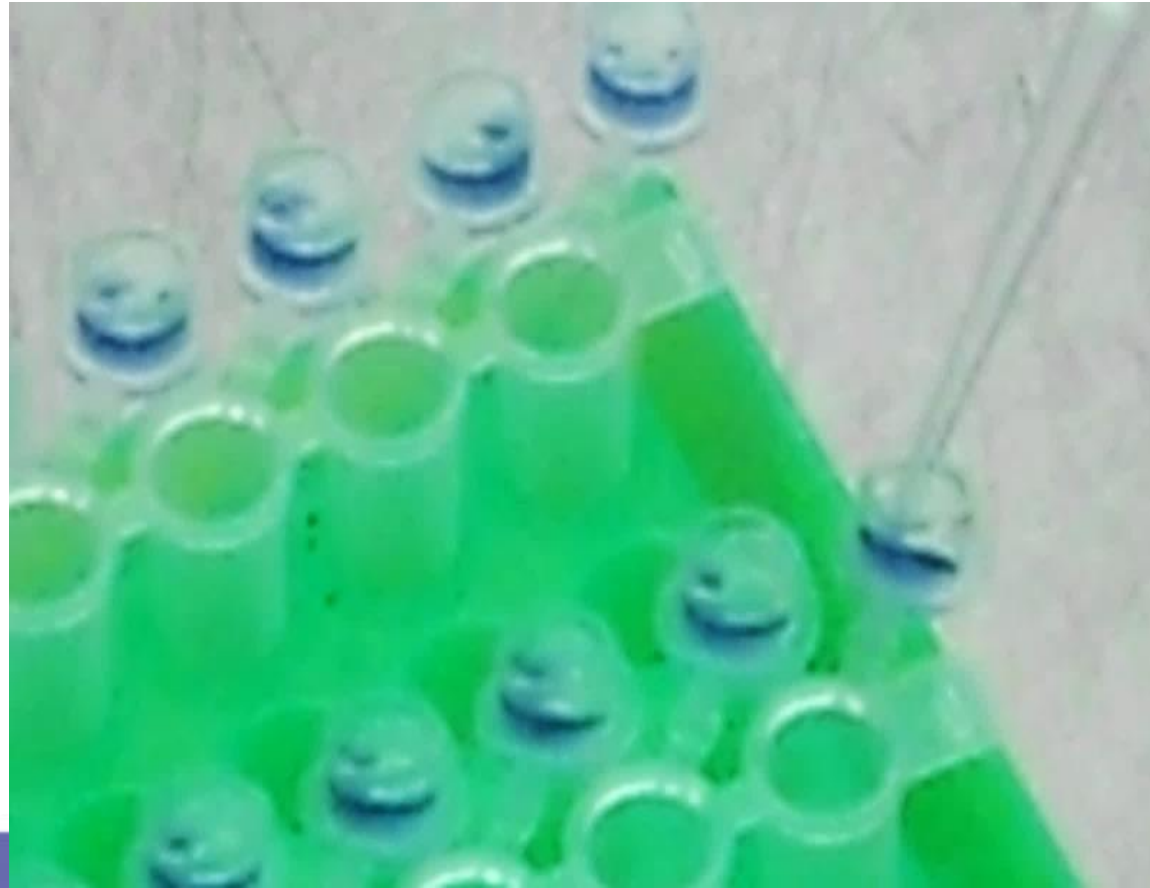


WHAT IS **dry format** RT-LAMP

- Loop-mediated Isothermal Amplification Method
 - uses single temperature (60-65°C) throughout the amplification period; uses two to three pairs of primers
- Dry LAMP uses vitrification technology where the reagents are changed from liquid to solid, unlike the original wet format LAMP.
- The disaccharide responsible for the vitrification holds the trapped biological molecules to retain their natural structures during drying (Teramoto *et.al* 2008) and prolongs shelf life of proteins in a dry state at room temp. **NO NEED FOR DRY ICE OR FREEZER for storage.**



- The LAMP mixture components are dried onto the lid of a single tube, so the reaction starts just after all the component (primer/enzyme/template) are mixed and incubated at the required temperature and time.



CONCEPTUAL FRAMEWORK

Sample
Collection

RNA
extraction

PCR
Amplification

Phylogenetic
analysis

RT-LAMP
Optimization and
Validation
*Wet and Dry
Format*

Transformation of
LAMP Dry format
into **TEST KITS**

RT-RPA
Optimization and
Validation
*Wet and Dry
Format*



METHODOLOGY



Backyard/Semi-commercial Pig Farms
(San Miguel, Bulacan/San Marcelino Zambales/
Munoz, NE/ Pangasinan)

NUMBER	TYPE
12	sow
16	mature boars
28	4-20 wks. old

PIGS

(vaccinated/nonvaccinated)

Sample Collection

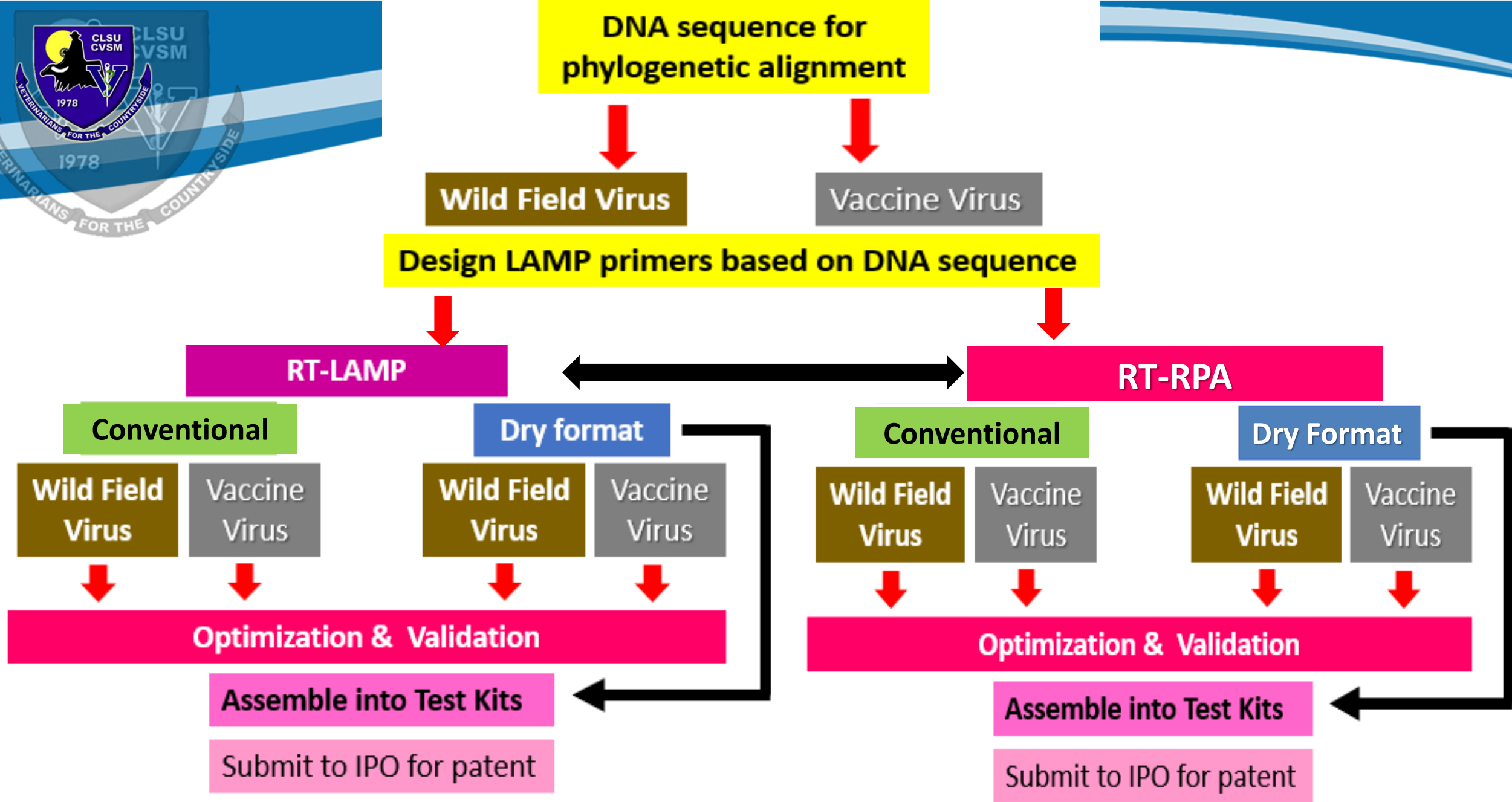
(whole blood, lungs, tonsils,
aborted fetus, semen)

Modified Live Vaccines for
CSF & PRRS (commonly used)

RNA extraction

RT-PCR

(target genes for CSF and PRRS viruses)



RESEARCH HIGHLIGHTS

Results

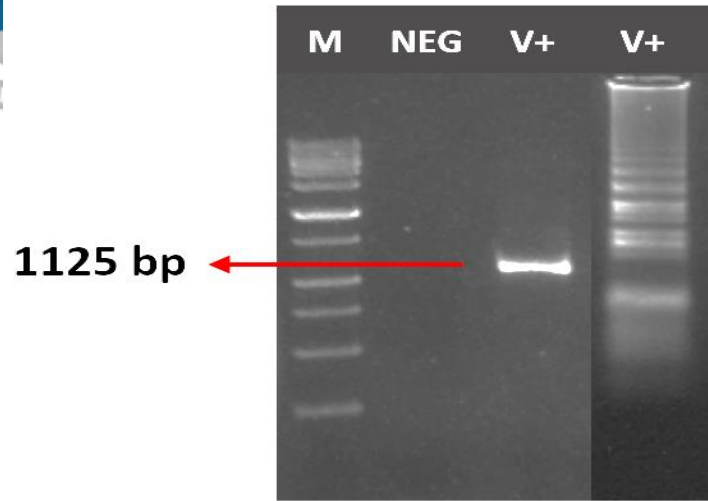


Fig.2 RT-PCR and RT-LAMP gel documentation of vaccine. From left - Gel image of RT-PCR product and LAMP product, respectively. **M** (marker or ladder); **NEG** (negative control); **V+** (RNA vaccine virus extract).

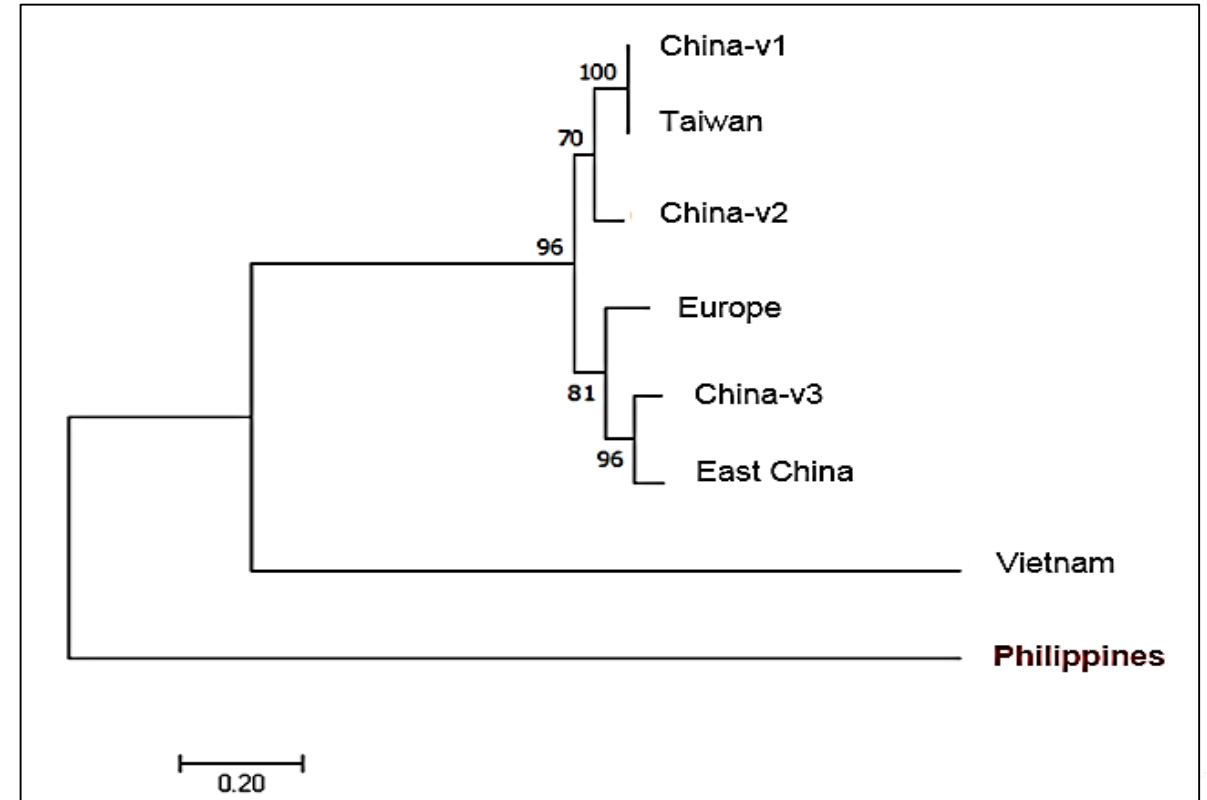


Fig.3 Phylogenetic tree diagram of the CSF vaccine (PigVax *C-strain*) in comparison with the CSF virus isolates from different countries.

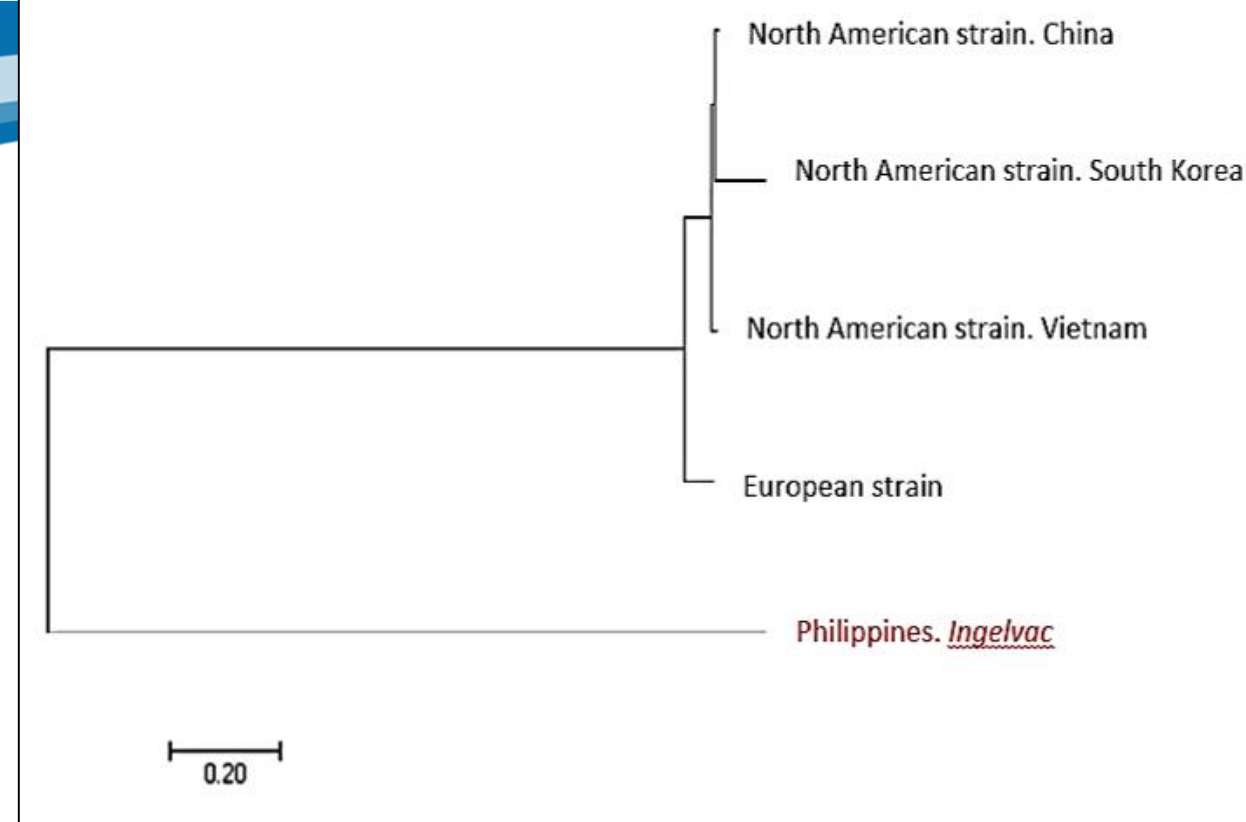


Fig.5 Phylogenetic tree diagram of PRRSV (Ingelvac™) compared with North American strain and European strain isolates from different countries.

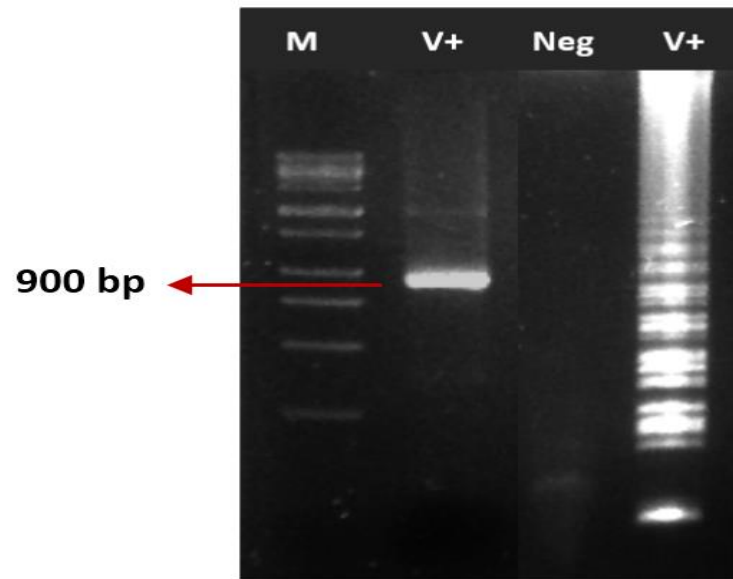
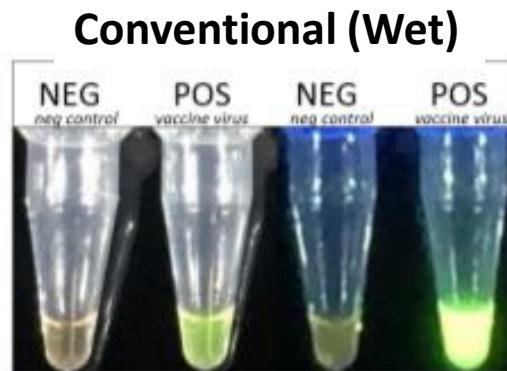
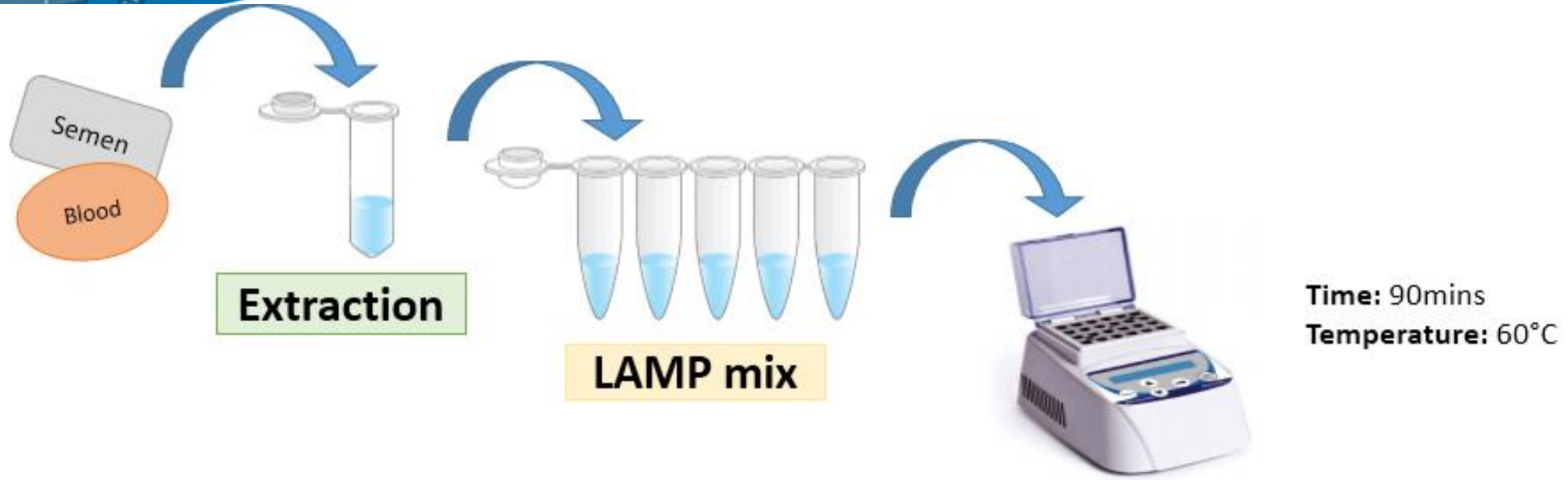


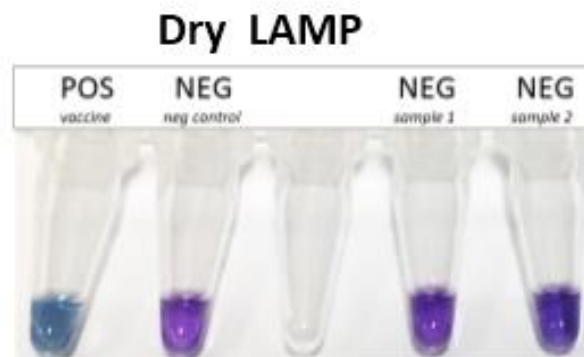
Fig.4 RT-PCR and RT-LAMP gel documentation of vaccine. From left - Gel image of RT-PCR product and LAMP product, respectively. **M** (marker or ladder; **V+** (RNA vaccine virus extract); **NEG** (negative control).

RT-LAMP Procedure



Naked eye
(+) green
(-) orange

UV light
(+) fluorescence



Naked Eye
(+) blue
(-) violet

Result

Incubation

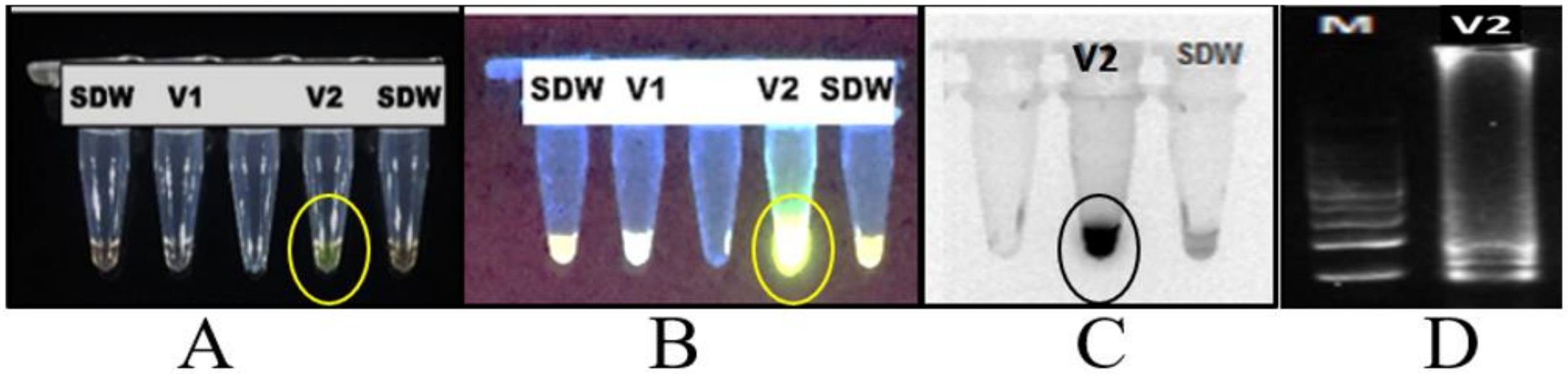


Fig.6 A-dye color change, B-fluorescence, C. Turbidity, D -Gel image. All LAMP primer sets were efficient in amplifying the CSFV target gene. V1 was a 3-wk old extract of a vaccine virus 3-week while V2 was a freshly extracted vaccine virus.

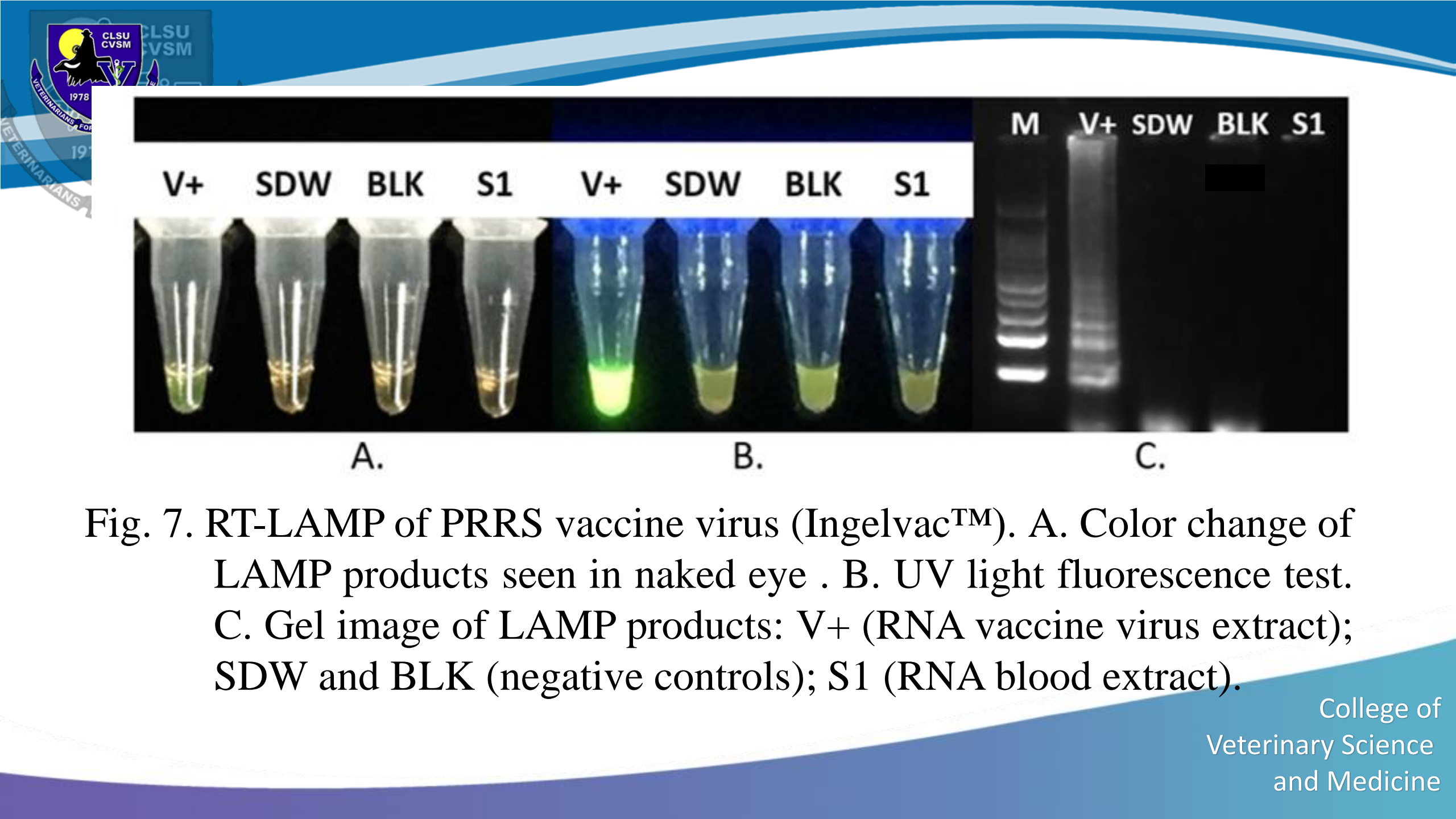
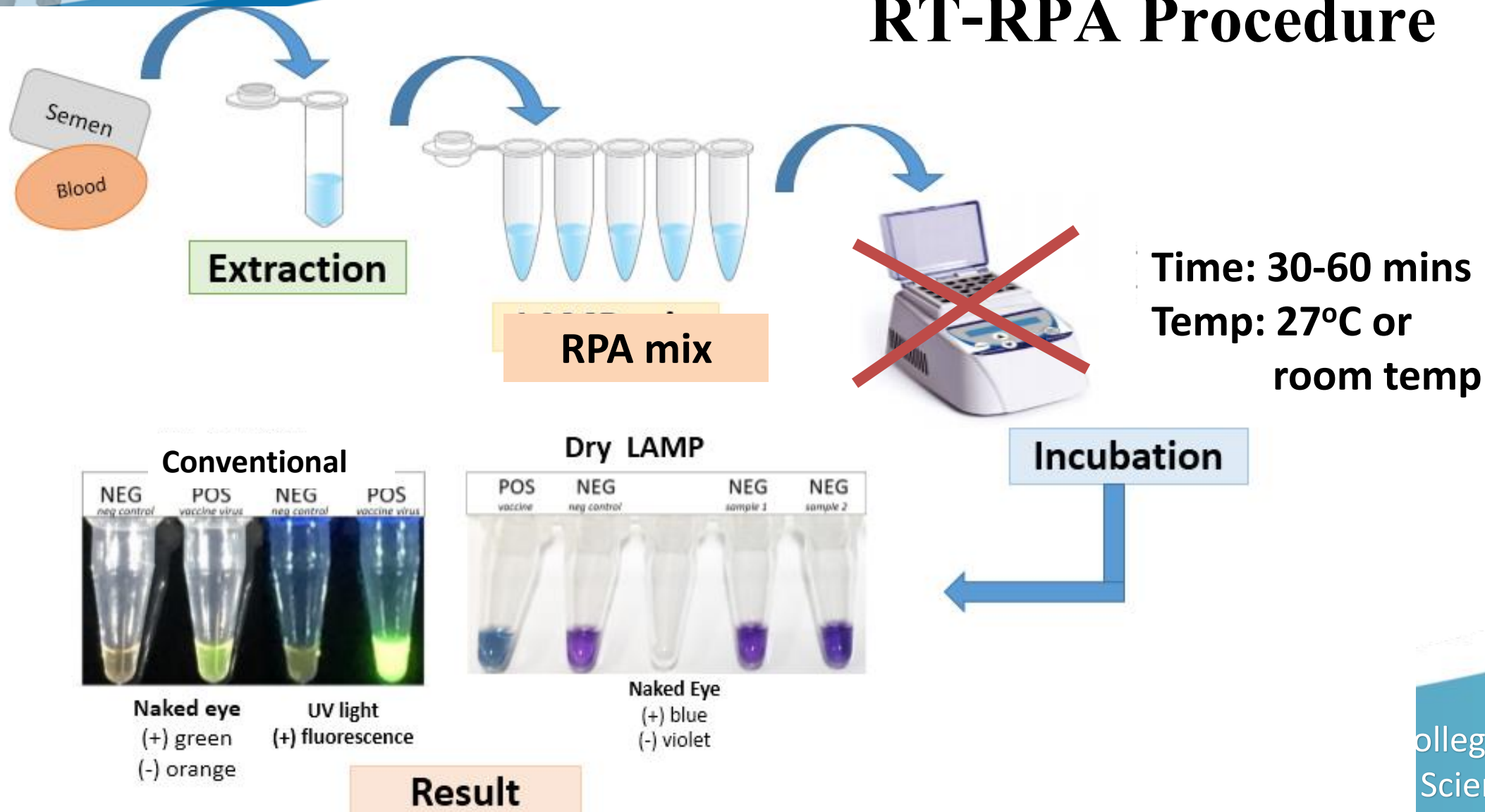


Fig. 7. RT-LAMP of PRRS vaccine virus (Ingelvac™). A. Color change of LAMP products seen in naked eye . B. UV light fluorescence test. C. Gel image of LAMP products: V+ (RNA vaccine virus extract); SDW and BLK (negative controls); S1 (RNA blood extract).



Fig 8. A. Dry LAMP products for detection of CSFV. (*From left: V1 and V2 from CSF vaccine, fresh and 1mo. old extract respectively; S1 and S2 from blood samples and SDW as negative control*). Color change from violet (negative) to blue (positive) indicates the result.

RT-RPA Procedure



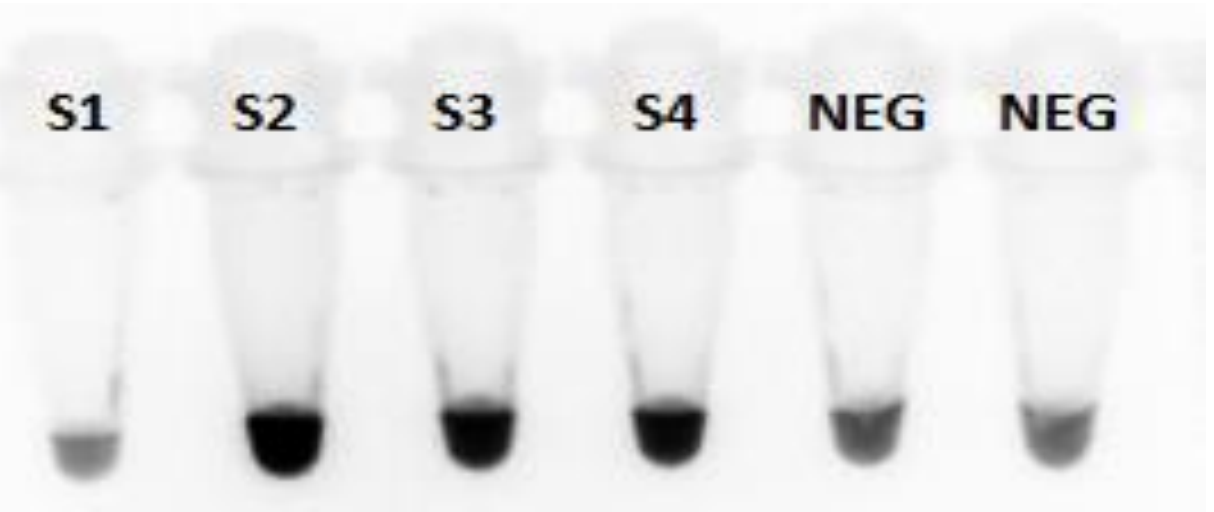


Fig 9. RPA detection of CSFV using conventional Sybr Green as dye indicator. *(Left)* RPA results in naked eye. *(Right)* RPA results under UV light. S1-S4 were blood samples collected within central Luzon while DDW and nontarget gene (PRRS vaccine virus) served as negative controls.

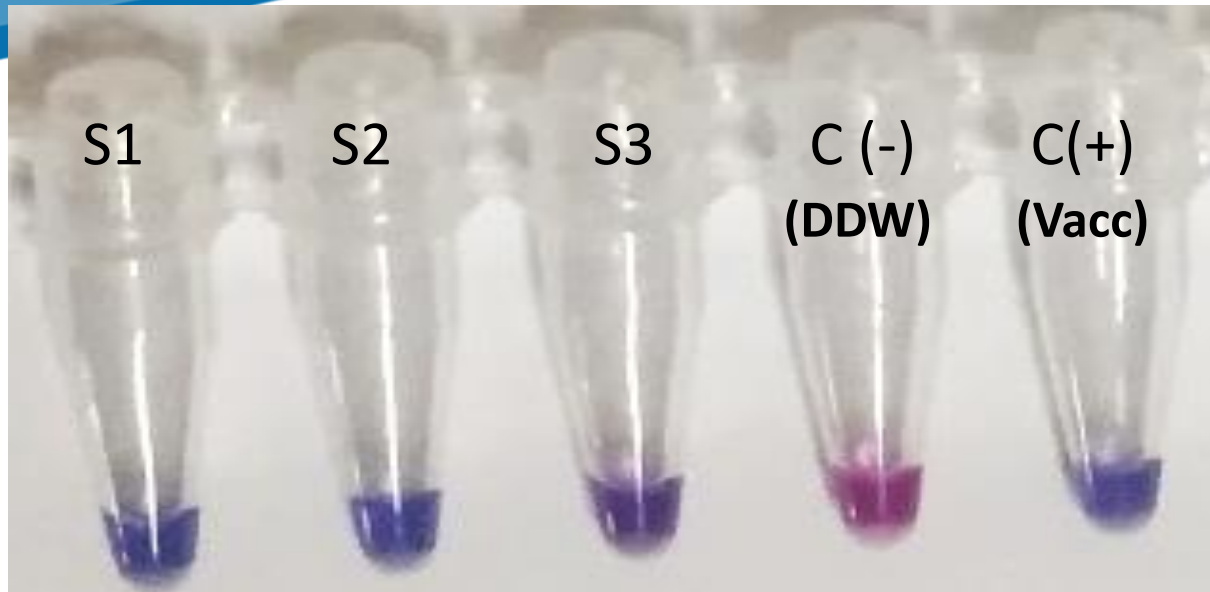


Fig 10. RPA detection of CSFV using Dry Format with CFI as dye indicator. *(Left)* RPA results in naked eye. S1-S3 were blood samples collected within central Luzon while DDW and CSFv served as negative and positive controls.



Surveillance Using Dry Format RT-LAMP



Collected Samples

Farm/Pen Location	Sample					
	Blood	Semen	Lungs	Tonsils	Spleen	Aborted Fetus
Bulacan	15	2	1	1	-	2
San Marcelino, Zambales	39	14	-	-	-	-
Science City of Munoz, Nueva Ecija	2	-	3	2	2	-
Talavera	-	2	-	-	-	-
Total	56	16	4	3	2	2

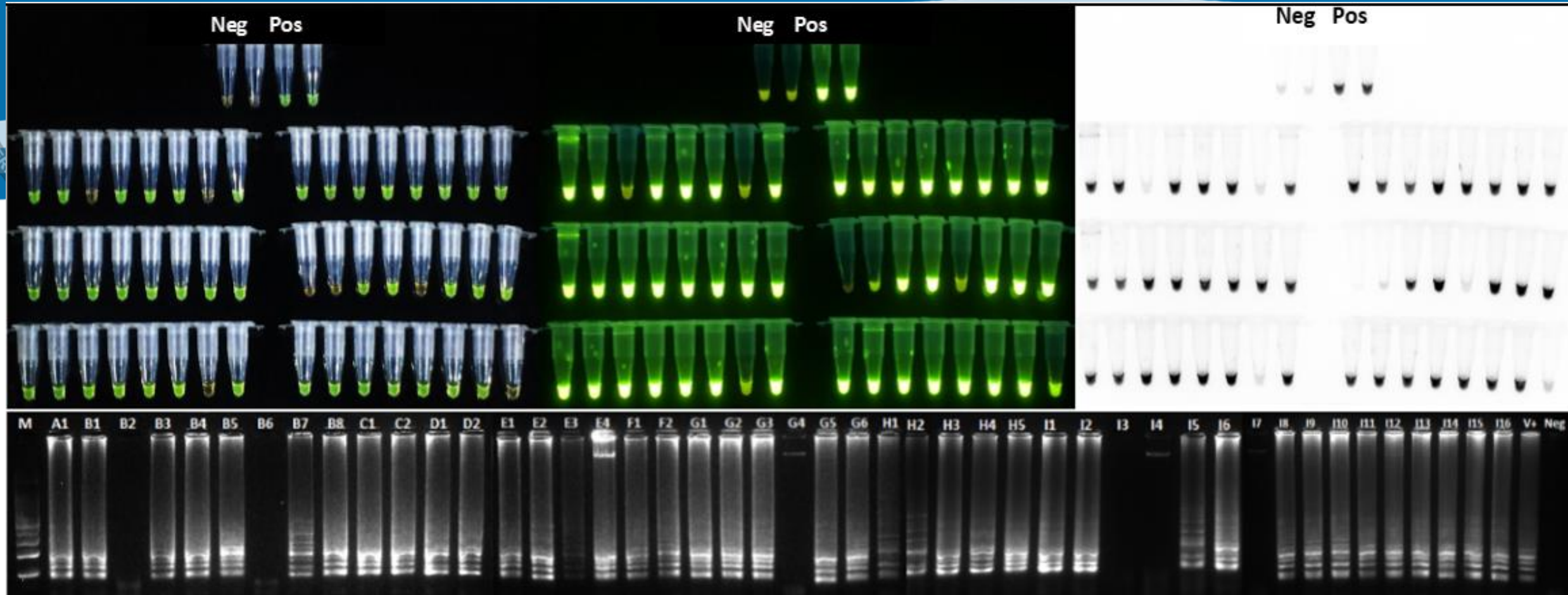


Fig.11 CSFV LAMP detection in blood samples collected from Nueva Ecija, Bulacan, Zambales and Pangasinan. **A.** Naked Eye (positive (+) showed by green color; negative (-) showed by orange color) **B.** UV light (positive (+) indicated by fluorescence) **C.** Turbidity test. (Positive (+) showed by presence of black precipitate. **D.** Gel image



Fig.12 CSFV Dry-LAMP detection in collected samples (lungs, tonsils, spleen and blood) from commercial and backyard farms in Pampanga and Bulacan.



Table 1. Dry LAMP results for CSFV detection for samples collected from slaughterhouses in Bulacan and Pampanga.

SAMPLES	POSITIVE		Total	% Distribution
	Backyard	Comm		
BLOOD	6	0	6	19%
LUNGS	4	0	4	13%
SPLEEN	6	6	12	38%
TONSILS	4	6	10	31%
TOTAL	20	12	32	

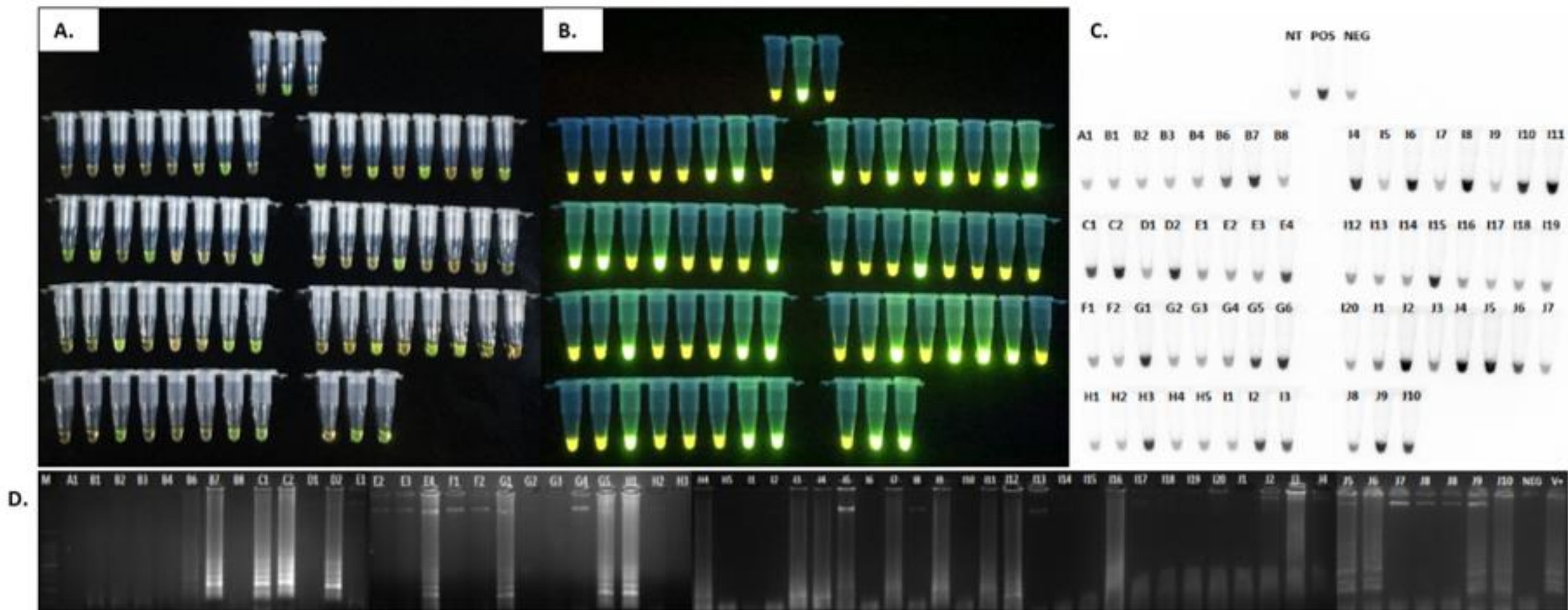




Fig.14 PRRSV Dry-LAMP detection in collected samples (lungs, tonsils, spleen and blood) from commercial and backyard farms in Pampanga and Bulacan.



Table 2. Dry LAMP results for PRRSV detection for samples collected from slaughterhouses in Bulacan and Pampanga.

	POSITIVE			
SAMPLES	Backyard	Comm	Total	% Distribution
BLOOD	1	0	1	4%
LUNGS	8	3	11	46%
SPLEEN	6	0	6	25%
TONSILS	3	3	6	25%
TOTAL	18	6	24	

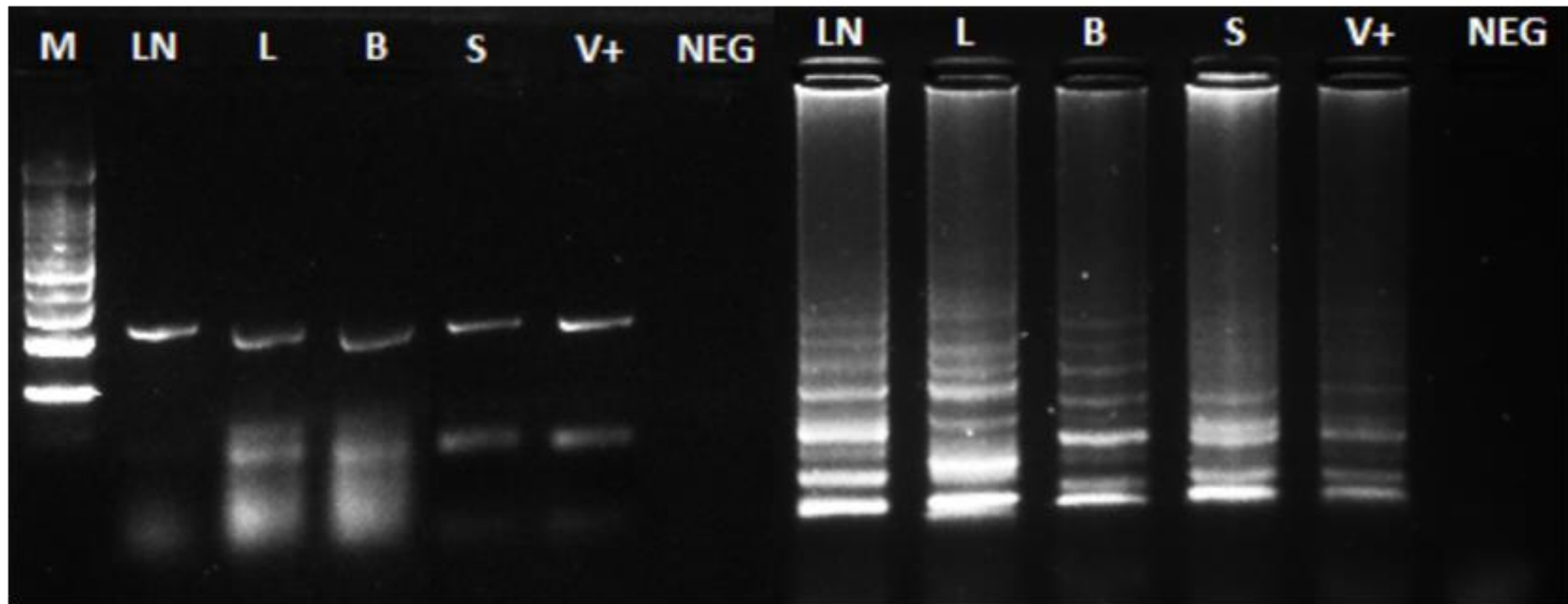


Fig.15 Gel image of amplified RT-PCR and RT-LAMP product of identified positive samples in PRRSV. (**LN**- Lymph nodes, **L**- lungs, **B**- Blood, **S**- Semen, **V+** vaccine, **NEG**- negative control)



NEEDS

- Wild field strain viruses for phylogenetic analysis, and for designing DIVA primers for both dry RT-LAMP and RT-RPA.
- Convert lab. protocols into handy test kit for future commercialization.
- BAI registration of the CSF and PRRS RT-LAMP & RT-RPA kits with DIVA capacity.



END