DEVELOPMENT OF RT-LAMP ASSAY ON *Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)* FIELD STRAIN IN THE PHILIPPINES

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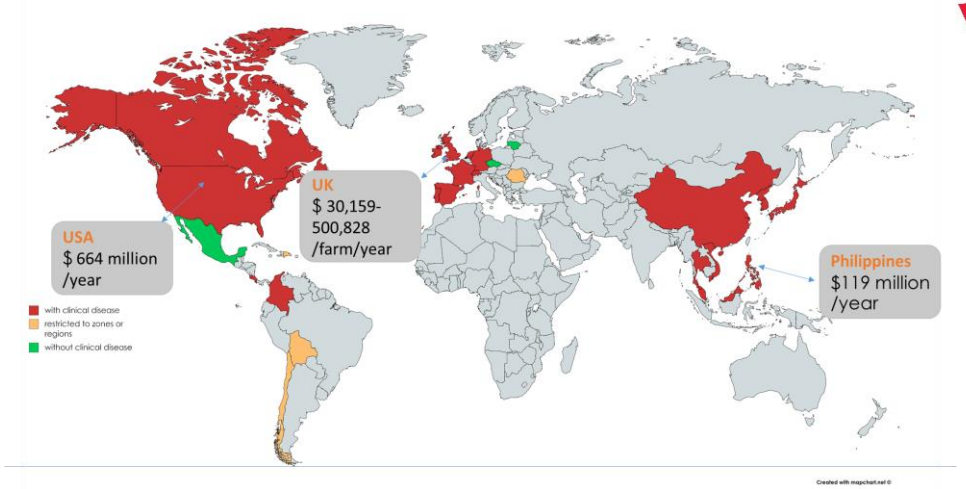
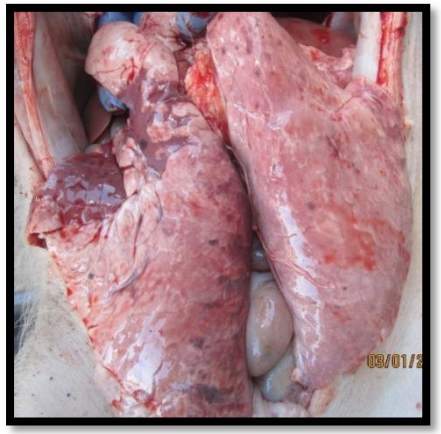
RATIONALE

- **PRRS as an economically important disease of swine worldwide**

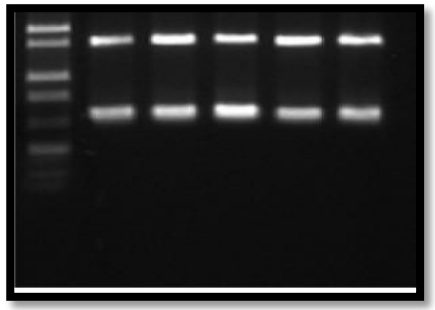


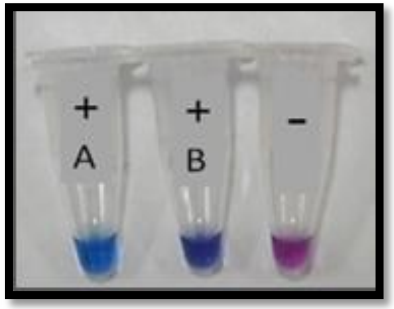
- **Diagnosis is often difficult.**



- **Diagnostic tests are available but vary in sensitivities and pose several limitations:**

Virus isolation	Serologic test
RT-PCR	RT-LAMP





RATIONALE

Dry vs. Wet RT-LAMP

CRITERIA	DRY	WET
LAMP premix dried	Yes	No
Positive result	Violet => blue	Brown => green
Re-open tubes to instill dye	No	Yes
Need for cold storage	No	Yes
Affected by inhibitors	No	Yes
Shelf-life	> 5 months	> 3 months

RATIONALE

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	/
/	/

RATIONALE

Why do we need to develop a new RT-LAMP assay for *PRRSV*?

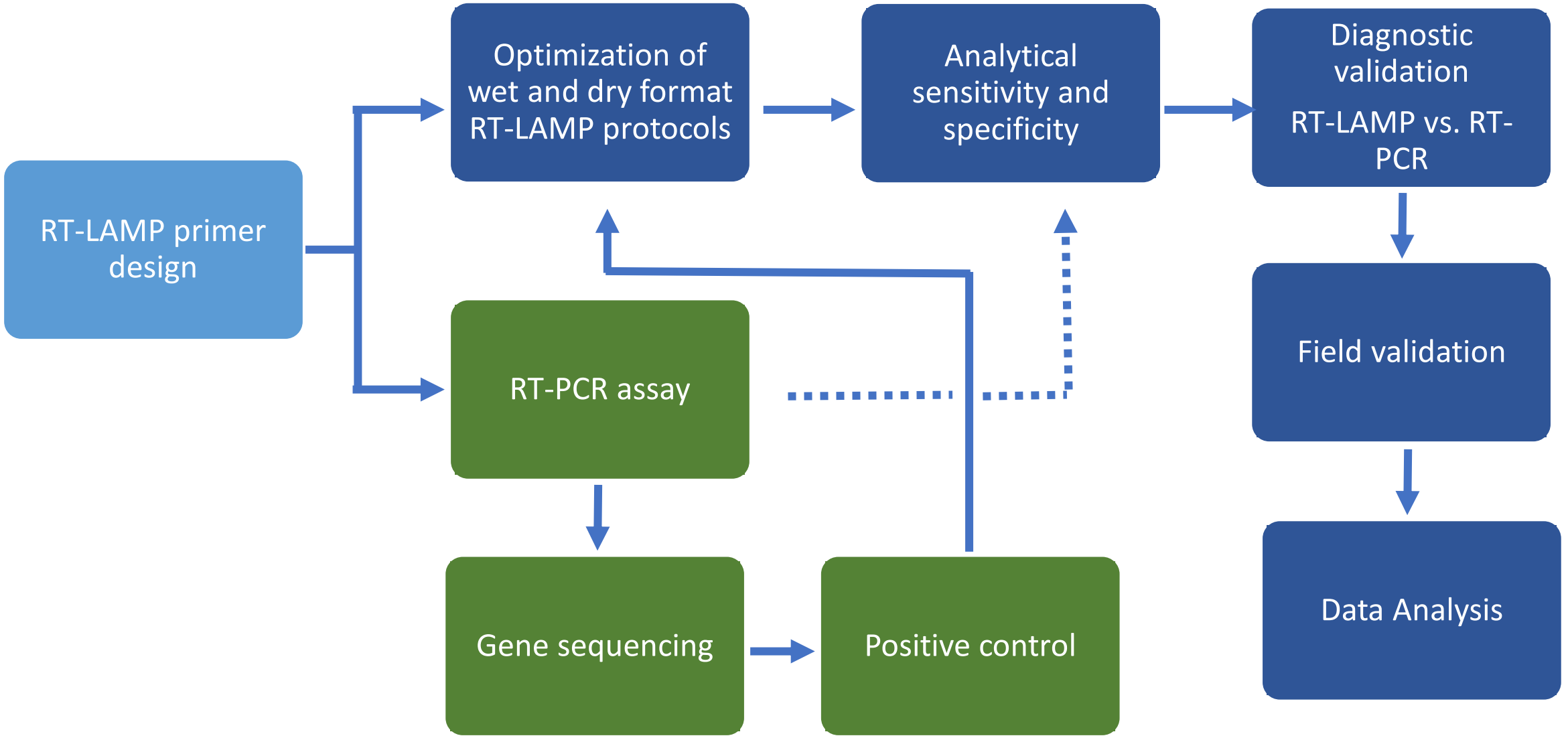
Published LAMP primers and protocols may not work in all laboratory settings.

RATIONALE

What could be the contributions of this RT-LAMP assay once developed?

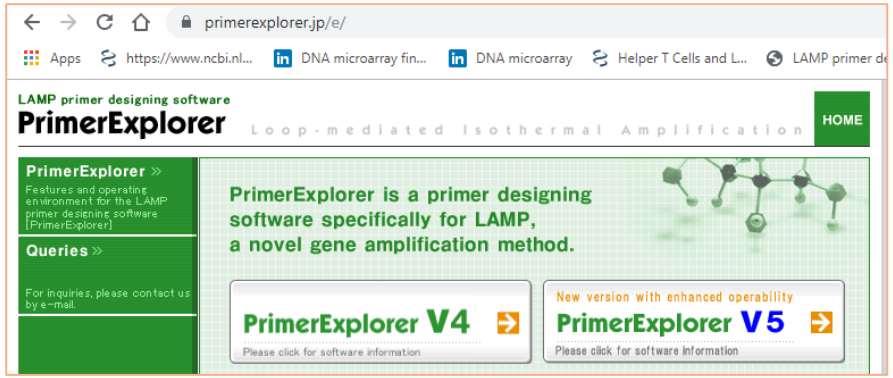
- ✓ Adopted by diagnostics laboratories
- ✓ Basis for developing an RT-LAMP assay kit for field diagnosis of PRRS
- ✓ Help farmers and veterinarians design control and prevention programs for PRRS in the farm

HOW ARE WE DOING IT?



HOW ARE WE DOING IT?

UPCVM-DA PL480 Project



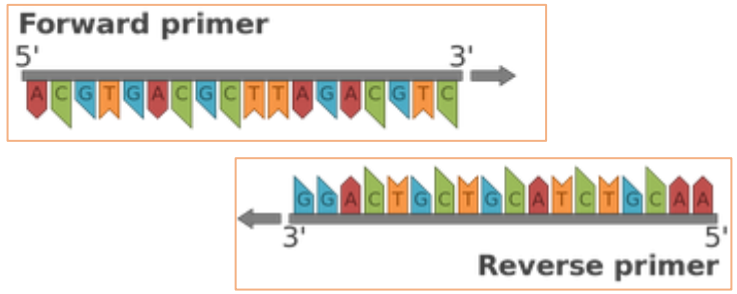
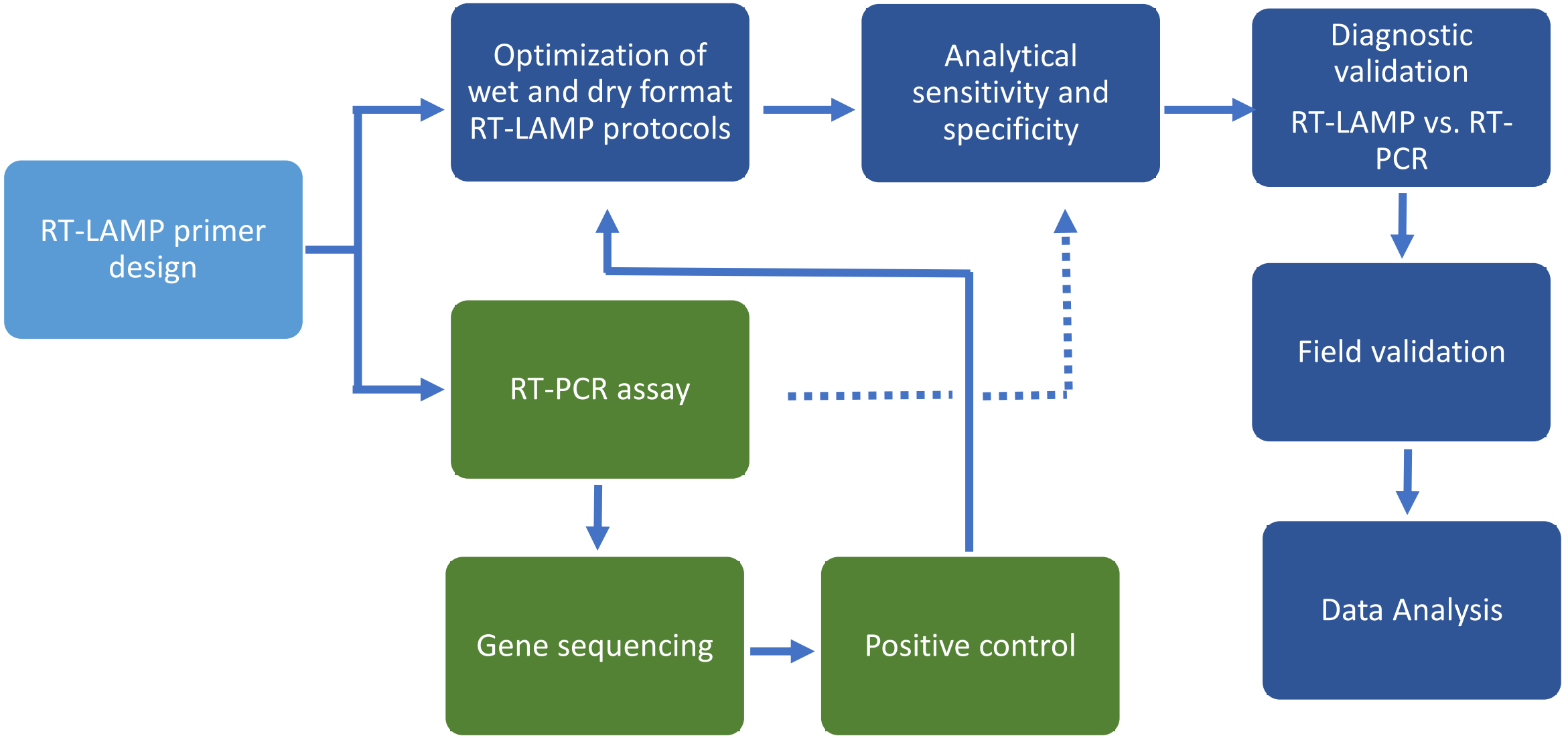


Table 2. The optimized primer sequences of *nsp2-B* epitope used in RT-PCR and RT-LAMP assay.

Primer	Length	Sequence (5'-3')
F3	19	GCC……TCGA
B3	20	AGC……..TGC
FIP	41	AAG……..AC-CAC……CGT
BIP	40	GTC…….....CAC-ACA……..TCC
F loop	20	GCG……TCTC
B loop	23	CCC……TGG

HOW ARE WE DOING IT?



OUR FINDINGS #1

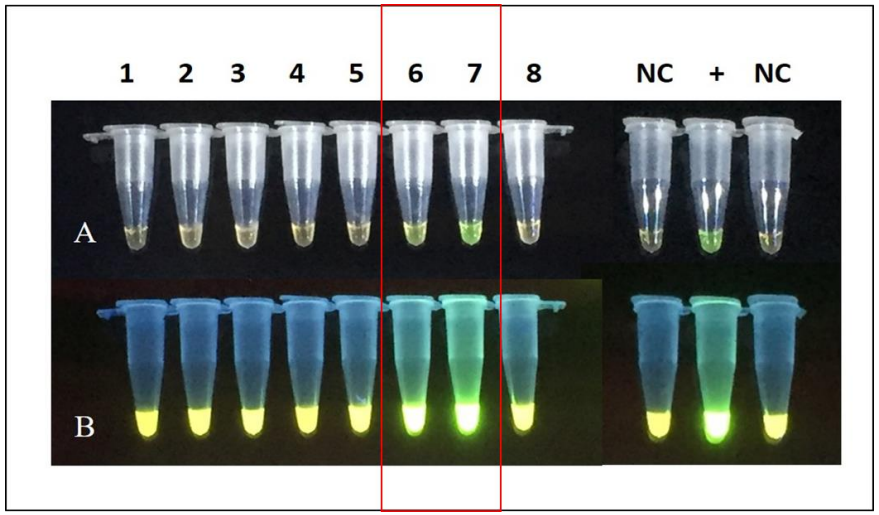
Table 2. The optimized primer sequences of *nsp2-B* epitope used in RT-PCR and RT-LAMP assay.

RT-PCR

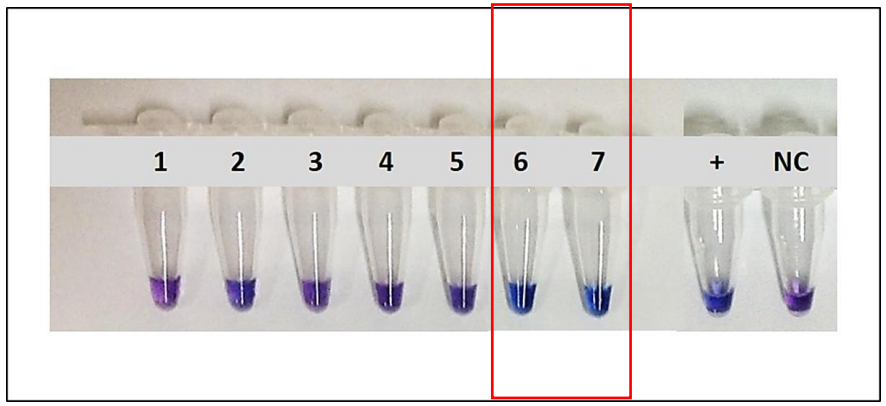
Primer	Length	Sequence (5'-3')
F3	19	GCC……TCGA
B3	20	AGC……..TGC
FIP	41	AAG……..AC-CAC……CGT
Loop Primers	40	GTC…….....CAC-ACA……..TCC
F loop	20	GCG……TCTC
B loop	23	CCC……TGG

- *3 pairs of primers optimized: outer, inner, loop*
- *Use of loop primers reduced amplification time from 1 hour to 30 minutes*

OUR FINDINGS #2



Wet format RT-LAMP



Dry format RT-LAMP

The results of wet RT-LAMP is in congruent with that of dry RT-LAMP.

OUR FINDINGS #3

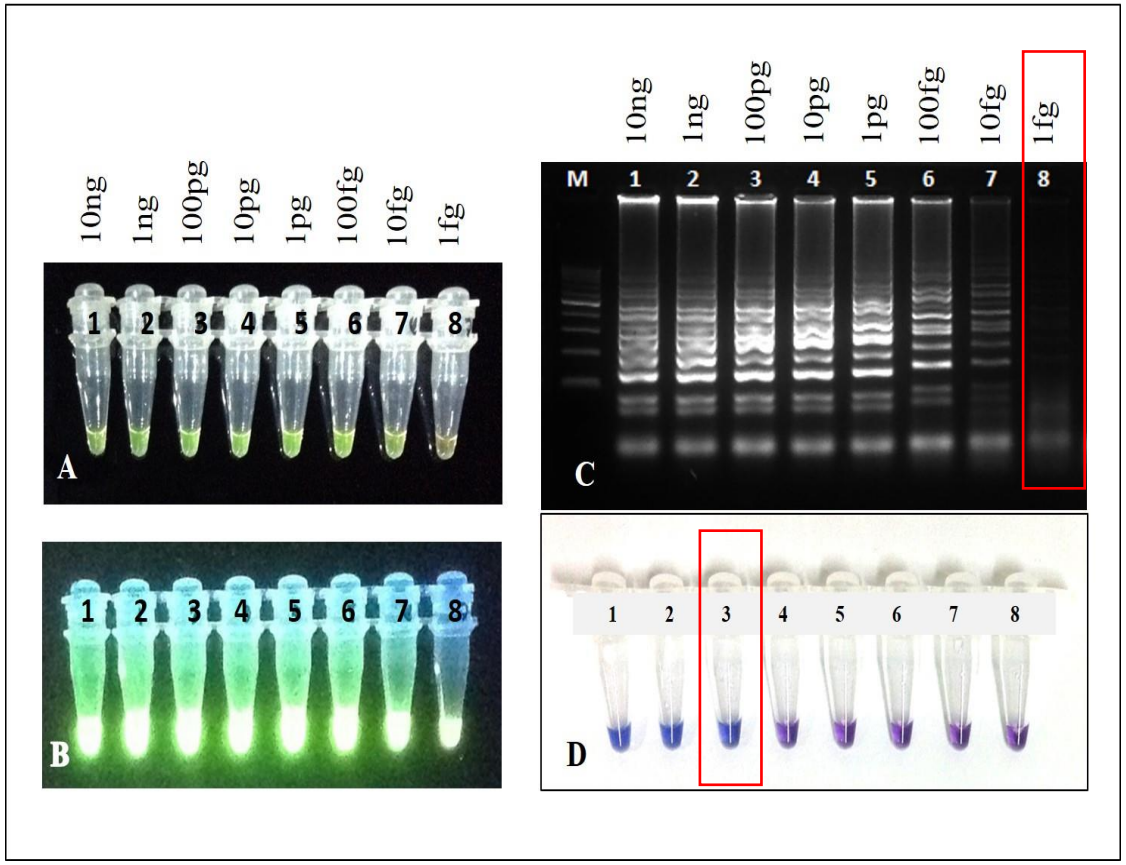
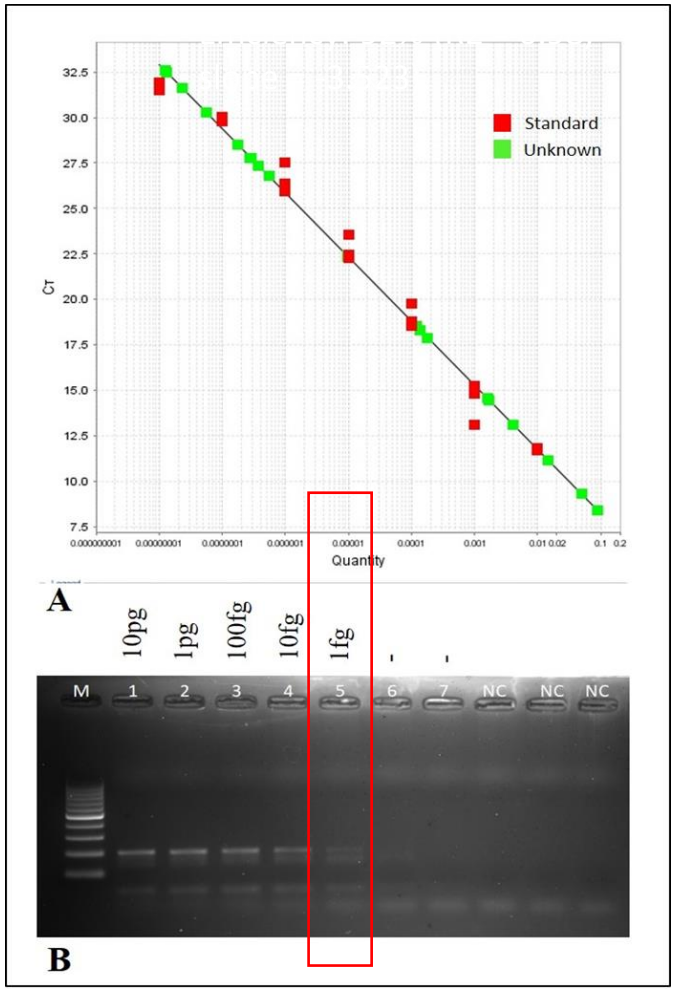
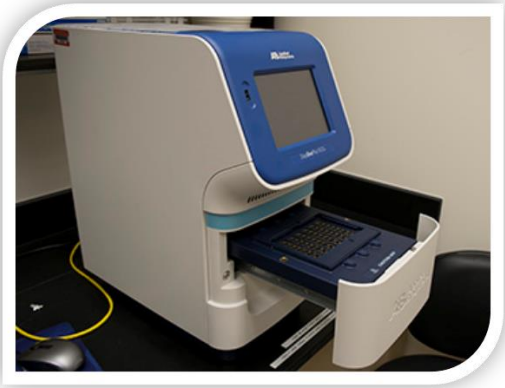


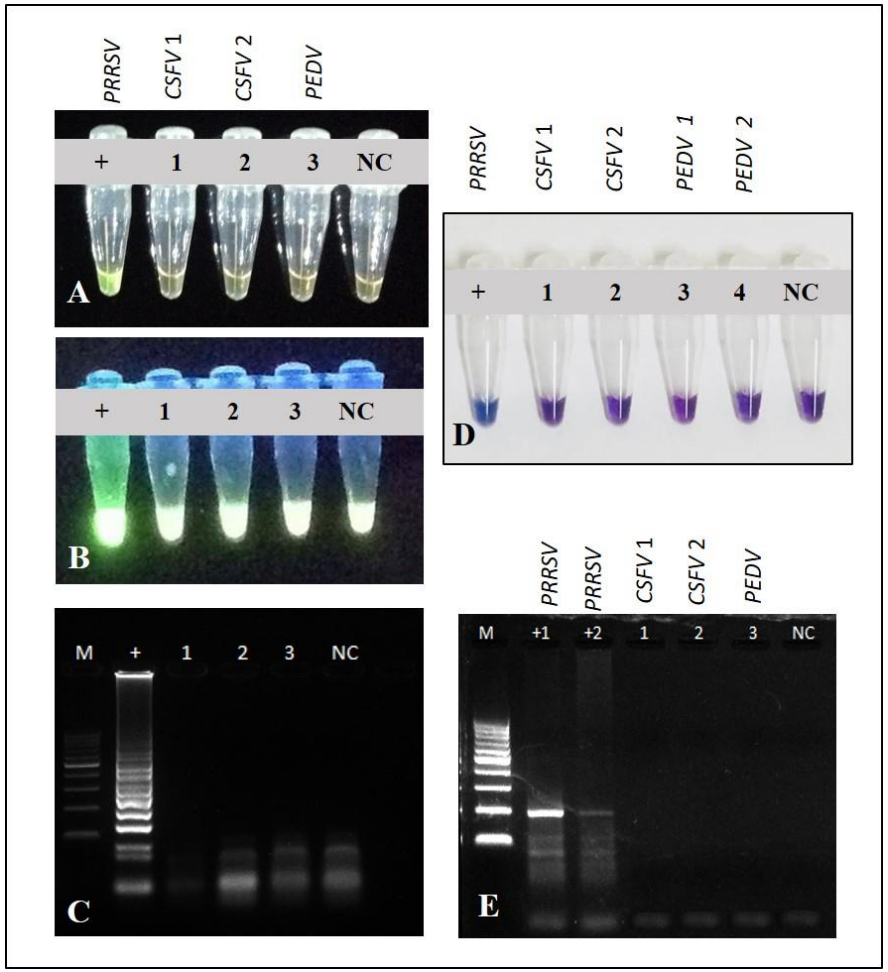
Figure 5. Analytical sensitivity of wet and dry format RT-LAMP vs. qRT-PCR using serially diluted viral RNAs.



Wet-format RT-LAMP and qRT-PCR has a lower limit of detection, i.e. more sensitive than dry-format RT-LAMP!



OUR FINDINGS #4



Wet and dry format RT-LAMP assays were specific for PRRSV.

Figure 7. Comparison of the analytical specificity of wet, dry format RT-LAMP and RT-PCR with PRRSV nsp2-specific primer set.

OUR FINDINGS #5

	RT-PCR		
	Positive	Negative	**Total**
RT-LAMP			
Positive	15	6	21
Negative	0	24	24
Total	15	30	45

Apparent Prevalence

RT-PCR = **33.33%**
(95%CI:44.73-48.56%)

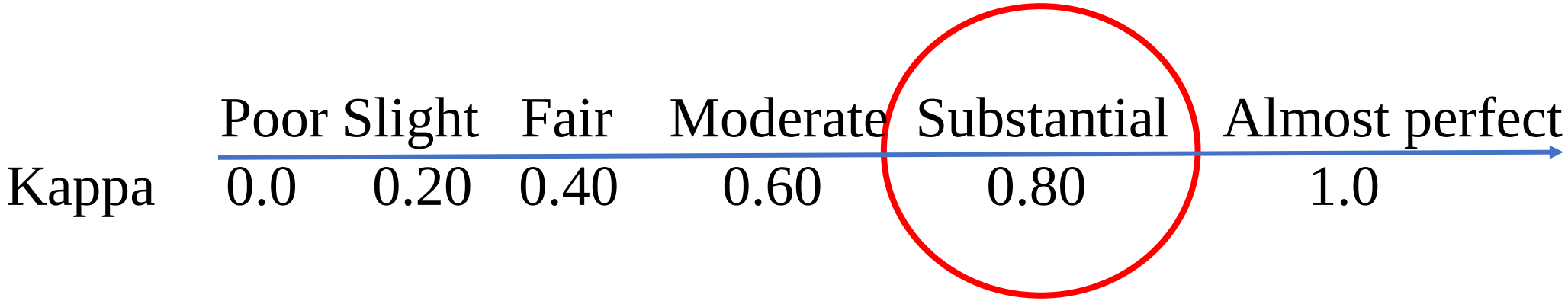
RT-LAMP = **47%**
(95% CI: 31.4-35.3%)

$$Apparent\ Prevalence\ (\%) = \frac{number\ of\ positive\ samples}{total\ number\ of\ samples} \times 100$$

OUR FINDINGS #6

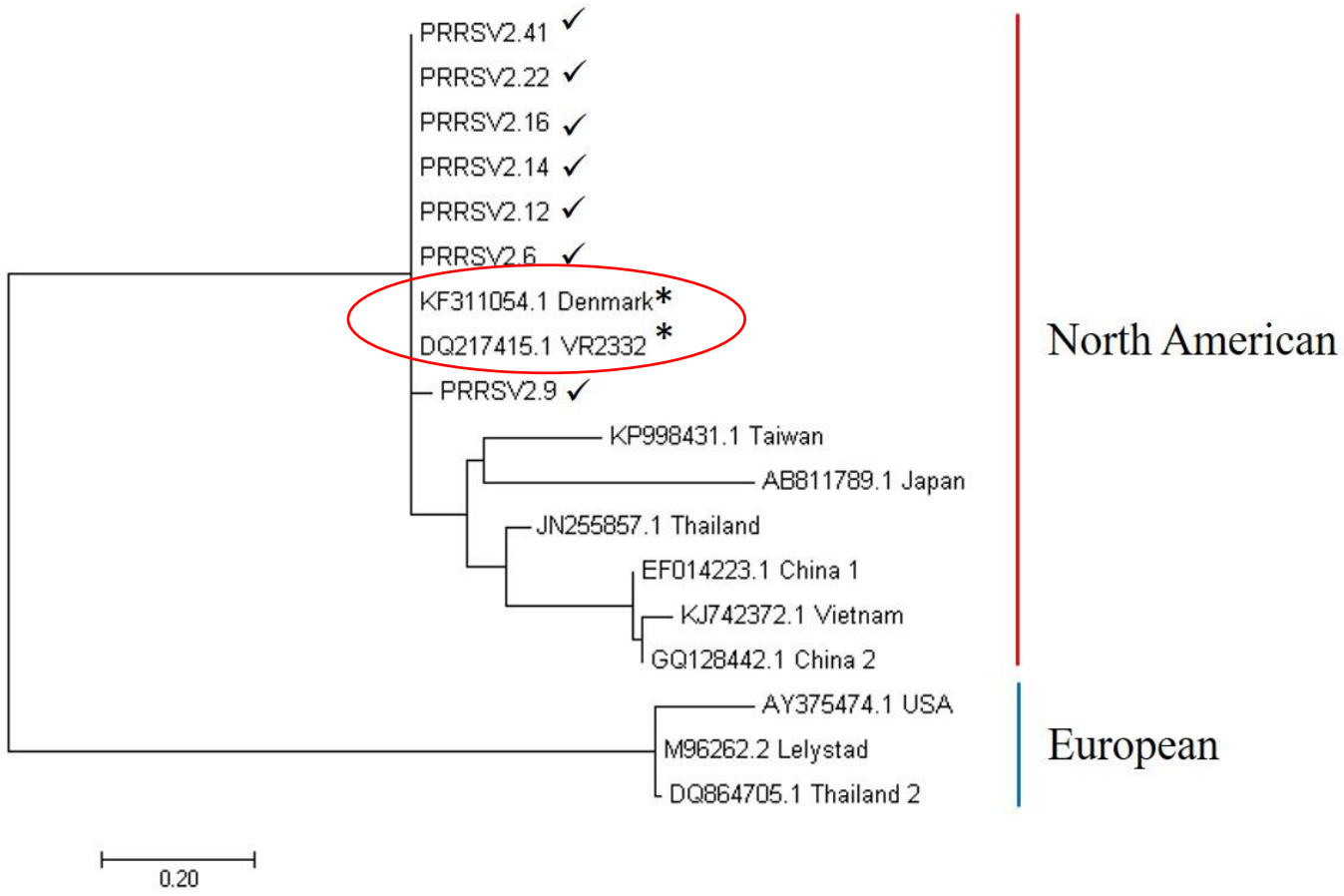
	RT-LAMP (wet and dry)
Sensitivity	100%
Specificity	80%
Kappa coefficient	0.73*

Kappa	Agreement
0-0.01	Less than chance agreement
0.01-0.20	Slight agreement
0.21-0.40	Fair agreement
0.41-0.60	Moderate agreement
0.61-0.80	Substantial agreement
0.81-0.99	Almost perfect agreement



OUR FINDINGS #7

Molecular phylogenetic analysis of *Porcine reproductive and respiratory syndrome virus* isolates based on partial *nsp2* nucleotide sequences



- ✓ *All seven isolates were of North American type, closely related to VR2332* and PRRSV2 DK-2011-30-6-27* strain*

SUMMARY, CONCLUSION AND RECOMMENDATION

- Wet and dry format RT-LAMP assays are optimized based on analytical sensitivity and specificity.
- Both formats are validated in the field=> 100% sensitive and 80% specific
- RT-LAMP and RT-PCR results have substantial agreement.
- Overall, this study was able to develop wet and dry format RT-LAMP assays for *PRRSV* that is robust and rapid, sensitive, specific, and can be used for field diagnosis.
- **Recommendations:**
  1. Further studies to improve the sensitivity of the developed dry format RT-LAMP assay
  2. Ways to reduce extraction time

ACKNOWLEDGEMENT

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- Department of Science and Technology-Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (DOST-PCAARRD) through the Graduate Research and Education Assistantship for Technology (GREAT) Program
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- Central Luzon State University

Partial sequence of *PRRSV* Laguna strain *nsp2* gene: Dr. Arman Parayao

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