# PEDv Rapid test

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## Introduction

Outbreaks of Porcine Epidemic Diarrhea virus (PEDv) cause significant economic losses, as well as negatively impacting animal welfare and productivity. Since the first outbreak in Canada in 2014, more than 200 cases of PED have been confirmed across numerous provinces. The virus causes diarrhea and vomiting in young pigs, and can be fatal. While PEDv is not known to pose any risk to human health or food safety, it is still a major concern for the industry. The virus is highly contagious and survives well in the environment, so strict biosecurity practices are key to stopping the spread of this pathogen. One important aspect of biosecurity is accurate and rapid disease detection – current standard PEDv detection depends on transportation of samples to diagnostic laboratories. The current test of choice is real-time reverse transcription PCR (rRT-PCR), which can be costly and time consuming as a result of the equipment, reagents, and expertise needed to run the testing.

> "The development of a rapid PEDv test kit will help producers identify potential risk on a more timely basis."

A reliable, cost-effective, and fast PEDv field diagnostic test kit is not currently available in Canada. Development and distribution of such a test kit will be valuable in reinforcing biosecurity measures and reducing the chance of this virus spreading. Loop-mediated isothermal amplification (LAMP) testing is an alternative nucleic acid amplification technique that does not require prohibitively expensive equipment or careful and extensive laboratory protocols. A rapid test for PEDv using reverse transcriptase LAMP (RT-LAMP) has been developed in the Phillipines. The Andali kit is a closed-tube system that contains target oligonucleotides (primers), a control DNA plasmid, and a premixed LAMP reagent. The kit also includes all necessary components for a simple nucleic extraction. The most expensive piece of equipment needed is a heating block to encourage the amplification reaction, which costs about $600 and is easily decontaminated. Total kit materials for a single test run add up to around $10, and the test can generate results in under an hour, with a colourimetric reaction indicating the presence of the virus. Though the Andali test kit has been validated in the Philipines, additional testing is needed to ensure that the primers used in the kit are viable for use to detect PEDv strains found in North America. Additionally, optimization of the kit was needed to ensure clear results, reducing ambiguity that may result during the interpretation of the colourimetric results.

What we did

Phase 1: To begin, we used an online database (BLAST – Global alignment) to ensure that PEDv spike protein sequence of several North American strains matched the primer sequence used in the Andali test (GenBank ID KM406181; 4126 base pairs). Luckily, the comparison suggested compatibility. To ensure that the test kit would generate the expected results, previously collected samples confirmed positive for PEDv using rRT-PCR were tested again using the kit. Some ambiguity was encountered as a result of the dyes used, so alternative dyes were selected for testing. Previously extracted viral RNA was used in the testing of these new components.

Phase 2: To evaluate the overall accuracy of the test kit following the modifications, actual field samples were collected from PEDv-positive barns. The collected samples were split and sent for testing at either a commercial laboratory or for testing at with the Andali kit. A total of 35 tests were conducted for comparison.
Phase 3: Following the testing in Phase 2, we set out to develop a new, user-friendly, step-by-step procedure for the modified kit. The instructions are being developed using plain language while also considering the requirements of diagnostic testing and surveillance programs to ensure that the results from the test kits are consistent and compatible. Alongside this guide, a short video is being produced to exhibit proper use of the kit and display expected results. These materials will not only be useful for training and instruction purposes, but also for the promotion of this kit to the target audience.

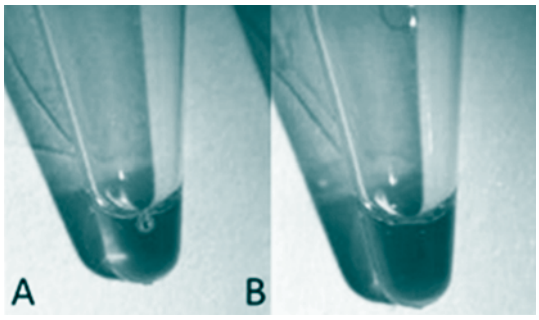


*Figure 1. Positive 'blue' (A) and negative 'purple' (B) RT-LAMP results from the Andali test kit.*

## What we found

Phase 1: Through comparison of the sequences of the PEDv spike protein found in North American strains and the sequence of the primer in the Andali test kit, it was found that there was a minimum 96.8% similarity, with most strains sharing >99% similarity with the primer. Testing with previous samples confirmed this finding, as the test gave expected results.

Phase 2: Analysis of the collected samples showed that the Andali test kit had a 100% positive predictive value (PPV) and had 90% total agreement with rRT-PCR analysis. The positive predictive value of a diagnostic test indicates the probability that a positive test is actually true – in this case, 100% PPV shows that no false positives were detected. The negative predictive value (NPV) of the Andali kit was 80%, which indicates that there is an 80% chance that a sample determined to be negative for the presence of PEDv came from a truly healthy pig. The results of this testing are considered adequate for the exploratory assessment of a potential PED outbreak. In some cases, observed ambiguities of the visual outcomes of the test kit made it difficult to differentiate between positive and negative tests, leading to false negatives (the sample was deemed positive by RT-PCR assessment but decided as negative in the field). As shown in Figure 1., the reference colour for positive is 'blue' and for negative is 'purple', though the test can range in resulting colours as seen in Figure 2. In this stage of testing, there was a 16.7% false negative rate.

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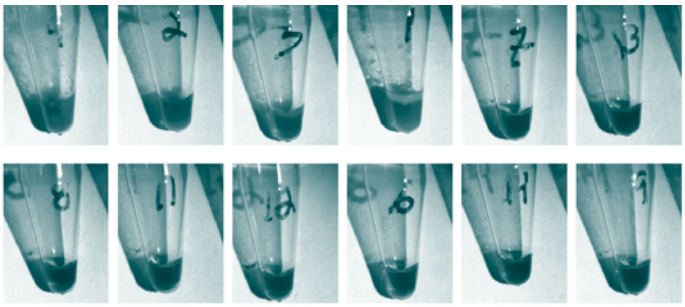

*Figure 2. Colour range of results from the Andali RT-LAMP test kit.*

*Figure 3. Results of field testing the modified test kit with SYBR Green as indicator dye as observed under normal light (A) and under UV light (B).*

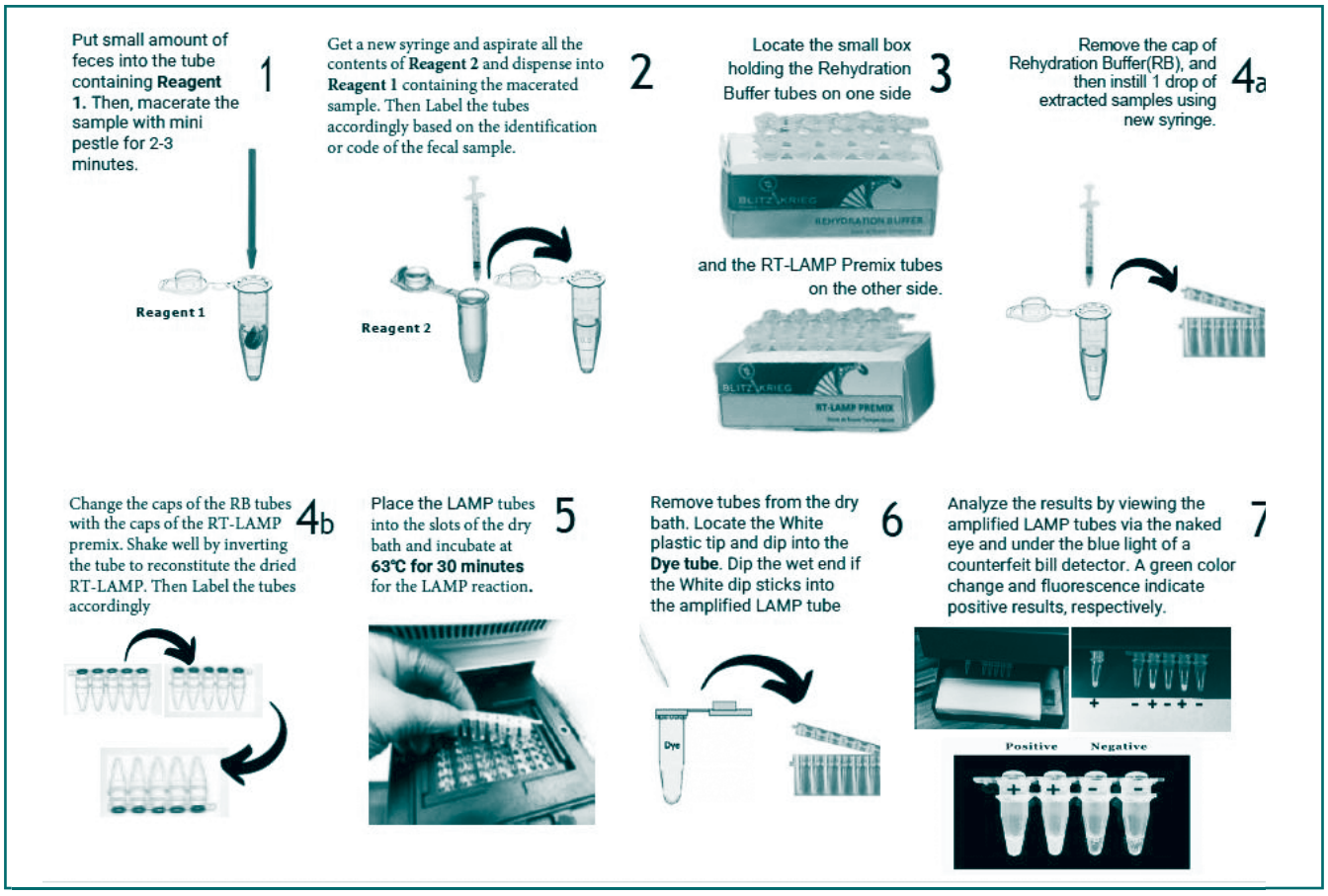


*Figure 4. Schematic diagram of the revised Users' Guide for the use of modified test kit.*

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In an effort to reduce ambiguity, different dyes were tested. Phenol red (PR) and SYBR green both had advantages and disadvantages. Use of PR ensured greater colour differentiation, but if an insufficient amount of sample was used or the extraction was not adequate, false negatives occurred. Use of SYBR green gave the added advantage of a fluorescent signal for positive tests, but the reagent requires careful environmental control and handling; using SYBR requires an additional piece of equipment, as UV light is needed to detect the fluorescent signal. Careful environmental handling is of the greatest concern for SYBR green, as exposure during transport can lead to false positives. To address this issue, changes to reagent formulation have been completed. Ultimately, it was determined that SYBR green gave the most accurate and unambiguous results, as the fluorescent reaction leaves little room for misinterpretation (Figure 3).

Phase 3: The existing Anadali user's guide has been updated based on North American conditions and requirements (Figure 4). Information on provincial surveillance program protocols has been incorporated into the guide and has also been used in the development of other instructional materials to ensure that adherence to sampling and testing requirements is maintained.

## What's next?

Based on the results of this study, the possibility of using the Andali test kit in Canada is clear. Porcine epidemic diarrhea continues to be a major concern in the North American swine industry, and it is paramount that we work to keep the virus under control. Though the kit was developed to test samples collected directly from animals, it can also be used to test critical sites and surfaces to ensure effective clean-up and decontamination, further supporting disease eradication. To continue the development and commercialization of this kit for use in North America, suitable permits need to be obtained and more testing needs to be done.

Future work on this project will focus on involving producers and veterinarians to test the kit in the field and provide feedback on user experience and the identification of results. To evaluate the efficacy of the updated kit in commercial situations before moving forward to commercialization, participation of commercial farms as demonstration sites will be needed to meet CFIA requirements for kit commercialization.