

Evaluation, optimization, and field validation of a rapid detection kit for Porcine Epidemic Diarrhea virus (PEDv)

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INSIGHT FOR PRODUCERS

Development of a rapid test kit for PEDv will help ensure that cases are caught quickly and that measures to prevent the spread of the virus are taken as soon as possible.

SUMMARY

Significant economic loss results from outbreaks of Porcine Epidemic Diarrhea (PED). Accurate and rapid detection of PEDv is essential for the implementation of control measures. A rapid PEDv test kit is needed for this purpose. Comparison of the DNA sequence targeted by the primers included in a PEDv test kit from the Philippines to sequences of North American PEDv strains showed suitability of these primers for detecting PEDv strains in the Canadian swine herd. This was confirmed in laboratory testing of 20 samples collected from PED-infected pigs. Test results showed visual interpretation of test kit results can be somewhat ambiguous for some types of samples, therefore can be further improved. While definitive results were obtained from most of the test samples, a few showed only slight visual differences between a positive result (indicated by a sky-blue colour) and negative results (indicated by purple or lighter blue colours). To avoid ambiguity, current work is focusing on using an alternative dyes to generate better colour differentiation between positive and negative results (yellow vs. pink/red colours). Further revisions such as reformulating reagents to dry format and modifying sample preparation procedures are also being done. The improved test kit will then be re-tested in the laboratory, where its sensitivity, specificity, and repeatability will also be assessed. Afterwards, the performance of the improved test kit will be validated by field testing in PED positive pig farms.

INTRODUCTION

Porcine Epidemic Diarrhea virus (PEDv) is a major pig disease currently facing the Canadian swine industry. Since the first outbreak in Canada in 2014, more than 200 cases of PED have been confirmed across several provinces, causing significant economic losses estimated to be about \$125,000 for a 1,000-sow herd. PED will remain a significant threat to the health and productivity of Canadian swine herds, therefore a reliable rapid diagnostic test kit will reinforce the biosecurity measures and be valuable in ensuring that herds are proactively protected against spread of this devastating disease.

Real-time reverse transcription polymerase chain reaction (rRT-PCR) is the method of choice for diagnosing PEDv infection. This testing is costly and requires sending the samples to a central analytical laboratory possessing the required equipment, delaying the rapid response needed to limit spread and impact of any emerging disease outbreak. Availability of a rapid and economical pen-side testing capability would be ideal, so that appropriate containment actions can be deployed immediately.

"Accurate and rapid detection of PEDv is essential for the implementation of control measures."

The Andali kit is based on Loop-Mediated Isothermal Amplification (LAMP), a nucleic acid amplification technique that can be used as an alternative to rRT-PCR without expensive or sophisticated equipment. The Andali test kit is a closed tube system that contains target oligonucleotides, control DNA plasmid and a LAMP reagent premix. It also involves a simple nucleic acid extraction process, with all components assembled into a kit. Using simple steps and hardware (i.e., a reusable heating block which costs about C\$600), a sample swab (or a representative composite sample) collected from the suspected site can be tested to confirm the presence or absence of PEDv in 30-45 minutes. This kit costs only \$10 CAD per test.

While the RT-LAMP test kit was initially validated by the original developers to detect PEDv strains endemic in their region (Philippines), further validation was needed to ensure that the test kit can accurately detect the various PEDv strains present in North America. Our current work, which builds on the findings from past and current projects is aimed at further improving the RT-LAMP-based kit to make it suitable for widespread use in the commercial swine industry Canada.

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EXPERIMENTAL METHODS

Phase 1

To ensure the Andali test kit was suitable for use with Canadian strains of PEDv, an online database (BLAST – Global alignment) was used to compare several North American PEDv sequences to the PEDv spike protein used in the test (GenBank ID KM406181; 4126 bp). After suggestion of compatibility, previously collected PEDv-positive samples from Manitoba and Saskatchewan were used to ensure the test kit would generate expected results.

Tests with the original Andali kit showed in some ambiguous results; to streamline identification, phenol red (PR) and SYBR Green were tested as alternative dyes. RNA extracted from several PEDv-positive fecal samples from Canadian barns was used as a target for testing new/alternative components and procedures.

Phase 2

The overall accuracy of the modified test kit was evaluated by evaluating actual field samples collected from confirmed PEDv-positive premises in Canada. Following routine sampling and diagnostic protocols, sub-samples from a PEDv site, of the collected material were sent to a commercial laboratory for PCR testing to confirm the status of the samples. The remaining sample materials were then tested using the modified test kit. A total of 35 tests were conducted, and results validated by comparing the same samples with PCR analysis.

Phase 3

In collaboration with the developer of the original test kit, a step-by-step procedure for proper use of the modified test kit was developed in plain language intended for target users, considering the requirements of diagnostic testing and surveillance programs implemented in various provinces ensuring results from using the test kit are consistent and compatible with existing surveillance programs. This user's guide, together with a short video clip as well as images and materials generated from the project will be used as aids for training the potential target users of the test kit on its proper use.

RESULTS

In silico primer assessment

The results of BLAST – Global alignment indicated that in most cases it had 99% or higher (with a minimum of 96.8%) similarity to respective sequences of PEDv strains from cases reported in Canada. These results from the in-silico analysis thus confirmed that the primers included in the original Andali PEDv test kit are suitable for detecting North American PEDv strains.

Kit evaluation and development

Results analyzed from 20 PEDv-positive and negative samples from Canadian pigs using RT-qPCR and the original Andali rapid test kit to detect PEDv showed the original rapid test kit had 100% Positive Predictive Value (PPV) and 90% accuracy or overall agreement with PCR readings. This was considered to be adequate for quick and exploratory assessment of a potential PED disease situation. However, there were observed ambiguities in the visual outcomes of the test kit which made it difficult to interpret the test results in some cases. As shown

in Figure 1, the reference color for the test kit result is 'blue' for positive (A) and 'purple' for negative tests (B); however, shown in Figure 2 are a few examples of the range of actual color of test kit results, some of which presented challenges when compared to the reference. Hence, some test results were misinterpreted, leading to 16.7% false-negative rate for this batch of samples.

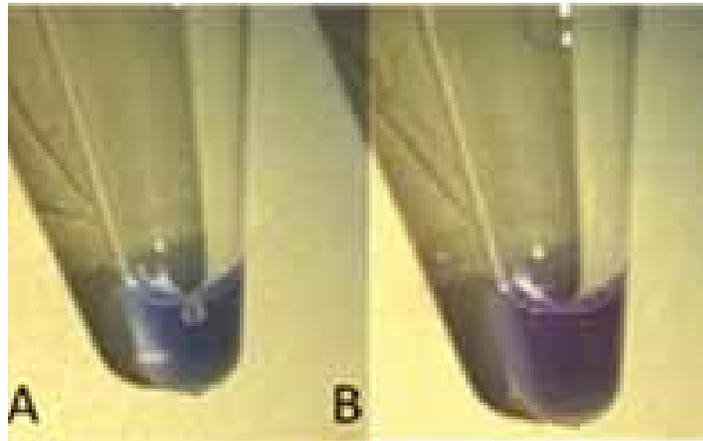


Figure 1. Positive 'blue' (A) and negative 'purple' (B) RT-LAMP results from the Andali test kit.

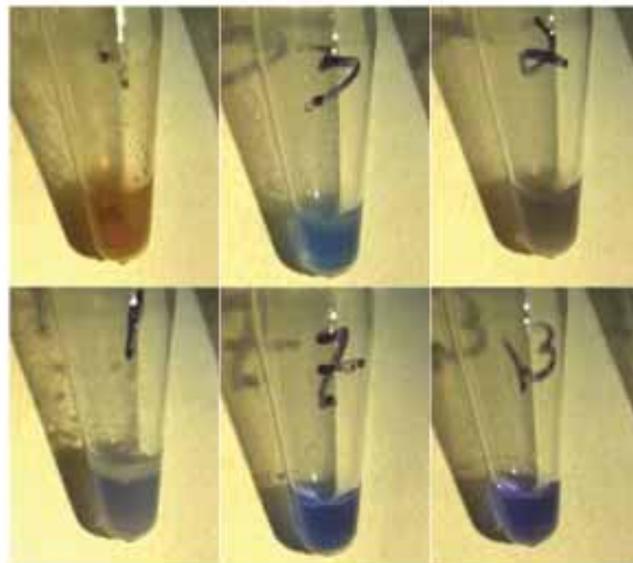


Figure 2. Colour range of results from the Andali RT-LAMP test kit.

"Using simple steps... to confirm the presence or absence of PEDv in 30-45 minutes. The kit costs only C\$10 per test."

Exploring alternative dyes to reduce the chance of any ambiguity, both PR and SYBR had advantages and disadvantages. Utilizing PR ensured greater colour differentiation but insufficient samples or imperfect extraction could result in false negatives. The SYBR Green version has the added advantage of a fluorescent signal to clearly distinguish positive test results, but requires careful environmental control and handling; it should be noted that this reagent requires a UV light source, which is an additional equipment which could add to the total cost of the test kit package.

Development of kit protocol and training materials

The existing user's guide of the original Andali test kit has been revised to reflect the conditions and requirements for its use in the Canadian swine industry. Information regarding the PED surveillance program protocols applied in various Canadian provinces has been collected and integrated into the other instructional and training materials to ensure adherence to province-specific sampling and testing requirements during the use of the revised PEDv test kit in Canada.

IMPLICATIONS

The possibility of using the Andali RT-LAMP kit in Canadian swine production is clear. To make this a reality, the developing lab based in the Philippines would need to work with a suitable Canadian entity or organization gain permits from government regulatory agencies (i.e., VDD, CFIA, etc.) and commercialize this test kit.

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