Detecting Porcine Coronaviruses PEDV, PDCoV and TGEV by Reverse Transcription Real-time PCR

Nardy Robben¹, Sandrine Moine², Robert Tebbbs³, Angela Burell⁴, Adam Allred⁵, Michelle Swinley⁶, Quoc Hoang⁷, Johnny Callahan⁸, Richard Conrad⁹

¹Thermo Fisher Scientific, Bleiswijk, Netherlands; ²Thermo Fisher Scientific, Austin, TX, USA; ³Thermo Fisher Scientific, Lissieu, France

ABSTRACT

We designed a reverse transcription-real-time PCR (RT-qPCR) assay to detect three porcine coronaviruses, porcine epidemic diathesis virus (PEDV), transmissible gastroenteritis virus (TGEV), and porcine deltacoronavirus (PDCoV), in a single reaction. This test can be performed on piglets in the immediate postnatal period, and can lead to quick actions, if needed, or additional examination of the intestinal lining often shows villous atrophy. Histological

INTRODUCTION

Porcine epidemic diathesis virus (PEDV), transmissible gastroenteritis virus (TGEV), and porcine deltacoronavirus (PDCoV) are viral infections that can cause gastrointestinal diseases in pigs. Clinical signs can include diarrhea, vomiting, dehydration and anorexia. Histological examination of the intestinal lining often shows villous atrophy. Current methods all of the shared in the detection of porcine coronaviruses and the importance of early detection has led to the need for regular environmental testing that includes checking trucks, barn walls, floors and incoming feed components to prevent the spread of new outbreaks in naive herds. Using environmental samples for detection of these pathogens will give information on herd level and can lead to quick actions, if needed, or additional sampling at the animal level to confirm. Environmental samples cannot be used to diagnose single animals in the herd.

SAMPLING & EXTRACTION

Environmental samples can be collected by several routes, including oral fluid, oral fluid, oral fluid, oral fluid, and oral fluid, and solid matter such as animal feed or feces. Samples are routinely broken off into a sealed tube containing 1 mL PBS after swabbing the surface of interest. Inclusion/exclusion testing of various oral fluid samples cannot be used to diagnose single animals in the herd.

ASSAY DESIGN

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RESULTS

Detection of Multiple Targets Simultaneously

PEDV detection (200x) with mixed target concentrations

PDV control (200x) with mixed target concentrations

PDCoV detection (200x) with mixed target concentrations

TGEV detection (200x) with mixed target concentrations

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Inclusion/Exclusion Testing of Various Field Samples

Table 2. Final Assay Designs

<table>
<thead>
<tr>
<th>Target</th>
<th>Target Sequence</th>
<th>Protocol</th>
<th>Blend</th>
<th>Target Group</th>
<th>Target Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEDV</td>
<td>5'-CCGACGCTGTGACCTGCTG-3'</td>
<td>TaqMan™</td>
<td>PDCoV</td>
<td>PEDV, PDCoV, TGEV, IPC</td>
<td>PEDV, PDCoV, TGEV, IPC</td>
</tr>
<tr>
<td>PDCoV</td>
<td>5'-GCATGAGTGGTGGTGTTAAAAA-3'</td>
<td>TaqMan™</td>
<td>TGEV</td>
<td>PEDV, PDCoV, TGEV, IPC</td>
<td>PEDV, PDCoV, TGEV, IPC</td>
</tr>
<tr>
<td>TGEV</td>
<td>5'-TTTGGGAGGATTTGTTTGCTTTAC-3'</td>
<td>TaqMan™</td>
<td>IPC</td>
<td>PEDV, PDCoV, TGEV, IPC</td>
<td>PEDV, PDCoV, TGEV, IPC</td>
</tr>
</tbody>
</table>

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Thermo Fisher Scientific R&D Team

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REFERENCES ON VIRUSES


TRADEMARKS/LICENSING

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