Analytical verification and use of a multiplex real time RT-PCR to identify Porcine Epidemic Diarrhea Virus, Transmissible Gastroenteritis Virus, and Porcine Deltacoronavirus

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Porcine Enteric Coronaviruses

• Cause significant economic losses for swine farmers
• US Swine industry provides $20 billion in annual gross income
• 3 porcine enteric CoVs are present in North America
Disease Significance and Impact

- Porcine Epidemic Diarrhea Virus (PEDV)
  - Severe diarrhea, vomiting and dehydration
- Porcine Deltacoronavirus (PDCoV)
  - Diarrhea
- Transmissible Gastroenteritis Virus (TGEV)
  - Severe diarrhea, vomiting and dehydration

Lost about 3.7 million pigs in the US during the “year of PEDV”
High Volume Testing

– Environmental testing to monitor and control the spread of disease within and between farms

– Clinical symptoms are similar among PEDV / PDCoV / TGEV (PDT)
  • Requires laboratory confirmation
Why a multiplex PCR?

- UMN Vet Diagnostic Lab 2014-2016 averaged: 54,500 PEDV rRT-PCRs per year
- Multiplexing saves reagents, supplies, equipment time/usage and technician time
Partnership and Reagents

- QIAGEN virotype PEDV/ TGEV/ PDCoV RT-PCR Reagents
  - PDT primers/probes (2 µL/rxn)
  - Positive PCR control

- Virotype mix 1 + IC (18 µL/rxn)

AAVLD strongly suggests the use of an internal control in all new PCR tests
# PDT Assay Parameters

<table>
<thead>
<tr>
<th>Target</th>
<th>Reporter</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEDV</td>
<td>FAM</td>
</tr>
<tr>
<td>PDCoV</td>
<td>Cy5</td>
</tr>
<tr>
<td>TGEV</td>
<td>JOE</td>
</tr>
<tr>
<td>Internal Control</td>
<td>TAMRA</td>
</tr>
<tr>
<td>Passive reference</td>
<td>ROX</td>
</tr>
</tbody>
</table>

## Temperature Parameters

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
<th># Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>50°C</td>
<td>10 min</td>
<td>1</td>
</tr>
<tr>
<td>95°C</td>
<td>2 min</td>
<td>1</td>
</tr>
<tr>
<td>95°C</td>
<td>5 sec</td>
<td>40</td>
</tr>
<tr>
<td>60°C</td>
<td>30 sec</td>
<td></td>
</tr>
</tbody>
</table>

*Fast! About 45 minutes*
Limit of Detection

U of MN

Ct Value vs. TCID50

QIAGEN

Found the PCR is sensitive to 1-10 viral copies per reaction for each pathogen
Amplification Efficiency

- The amount of PCR product increase after each cycle

\[
E_{PEDV} = -1 + 10^{-1/slope} = -1 + 10^{-1/-3.5107} = -1 + 10^{0.2848} = 0.9965
\]

\[
E_{PDCoV} = -1 + 10^{-1/slope} = -1 + 10^{-1/-3.3646} = -1 + 10^{0.2972} = 0.9825
\]

\[
E_{TGEV} = -1 + 10^{-1/slope} = -1 + 10^{-1/-3.318} = -1 + 10^{0.3014} = 1.0017
\]

- PEDV: 99.7%
- PDCoV: 98.3%
- TGEV: 100%
Excellent repeatability:
<4% in all cases
Analytical Specificity

Confirmed no cross-reactivity with 56 bacterial and viral isolates

- Porcine cytomegalovirus
- North American Porcine Reproductive and Respiratory Syndrome
- European Porcine Reproductive and Respiratory Syndrome
- Pseudorabies virus
- Swine Influenza virus H1
- Swine Influenza virus H2
- Swine Influenza virus H3
- Porcine Respiratory Coronavirus
- Porcine Adenovirus
- Porcine Circovirus Type I
- Porcine Circovirus Type II
- Picorna virus (Seneca Valley Virus)
- Porcine Rotavirus Group B
- Porcine Rotavirus Group C
- Porcine Lymphotropic Gamma Herpes Virus 1
- Porcine Lymphotropic Gamma Herpes Virus 2
- Porcine Hokovirus
- Beta-hemolytic Escherichia coli
- Clostridium perfringens type C
- Salmonella typhimurium
- Brachyspira hampsonii Colon A
- Brachyspira hampsonii Colon B
- Brachyspira hyodysenteriae
- Brachyspira pilosicoli
- Brachyspira murdochii
- Brachyspira intermedia
- Brachyspira innocens
- Actinobacillus suis
- +
Diagnostic Comparison

- 360 samples compared between the UMN in-house assays and Qiagen’s PDT triplex
  - 127 feces / fecal swabs
  - 99 intestines
  - 92 oral fluids
  - 42 environmental samples
Correlation

PDT triplex compared to our previous, in-house PCRs:

98%
98%
100%
Diagnostic Sensitivity and Specificity

Diagnostic Sensitivity:  
(The ability to detect true positives)

<table>
<thead>
<tr>
<th>Virus</th>
<th>PEDV</th>
<th>PDCoV</th>
<th>TGEV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>87%</td>
<td>92%</td>
<td>100%</td>
</tr>
</tbody>
</table>

True positives  
(true positives + false negatives)

Diagnostic Specificity:  
(The ability to detect true negatives)

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<tbody>
<tr>
<td></td>
<td>99%</td>
<td>98%</td>
<td>100%</td>
</tr>
</tbody>
</table>

True negatives  
(true negatives + false positives)
Diagnostic Sensitivity and Specificity

Diagnostic Sensitivity: (The ability to detect true positives)
- PEDV 87%
- PDCoV 92%
- TGEV 100%

Diagnostic Specificity: (The ability to detect true negatives)
- PEDV 99%
- PDCoV 98%
- TGEV 100%

Disagreements between 11 oral fluid samples (positive with old method but negative with PDT triplex)
Study Limitations

- Diagnostic sensitivity and specificity calculations assume our previous in-house assays are the “gold standard”
- TGEV positive samples rare – all TGEV comparison samples either negative or strongly positive
- Oral fluid discrepancies in 11 samples; unknown which assay gave the true result for these
Conclusions

- QIAGEN’s PDT triplex is in-use at the U of MN VDL
  - Faster
    - Time to results <1 hr
  - Better
    - Meets acceptance criteria
  - Cheaper
    - Saves money via reduced reagents, technician time and equipment use
Thank you