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

Swine Enteric Coronavirus Diseases (SECD)

- 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

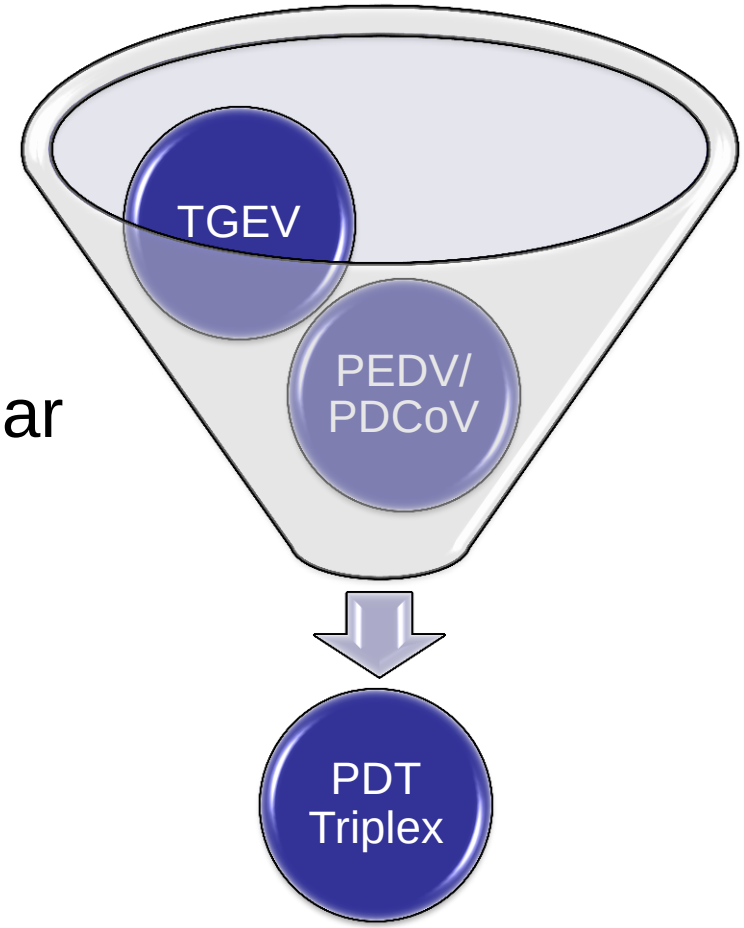
Why a multiplex PCR?

- 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

Target	Reporter
PEDV	FAM
PDCoV	Cy5
TGEV	JOE
Internal Control	TAMRA
Passive reference	ROX

Temperature	Time	# Cycles
50°C	10 min	1
95°C	2 min	1
95°C	5 sec	40
60°C	30 sec	

Fast!
About 45 minutes

Limit of Detection

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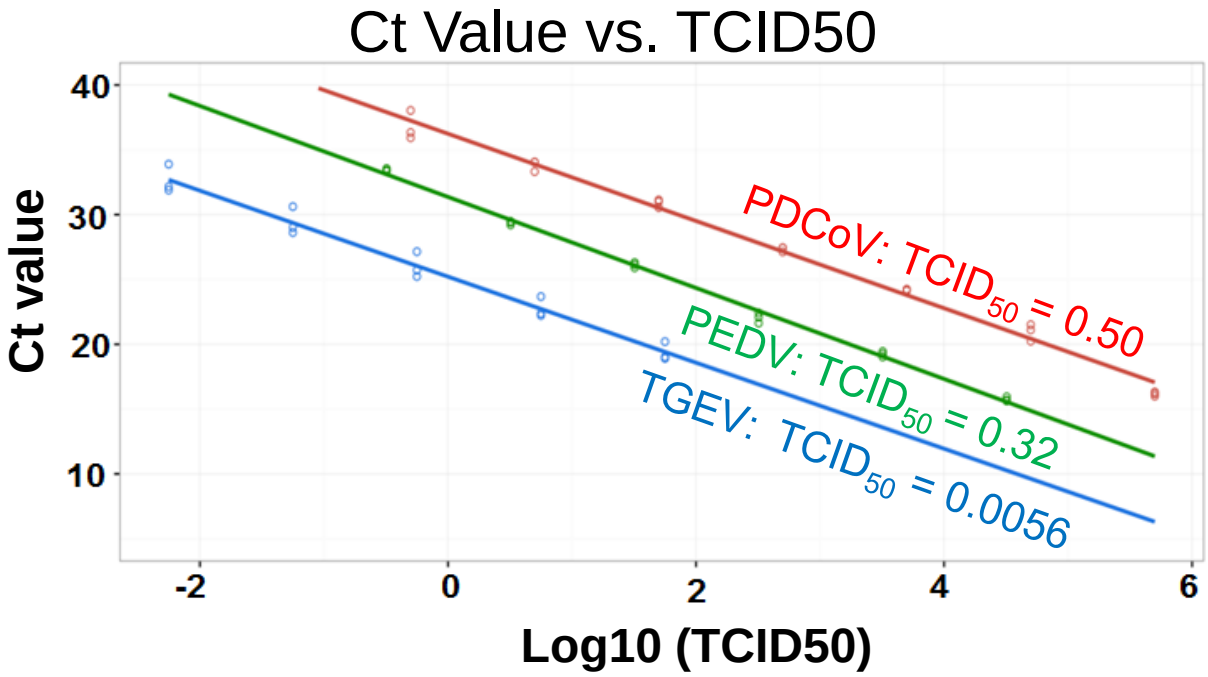


QIAGEN

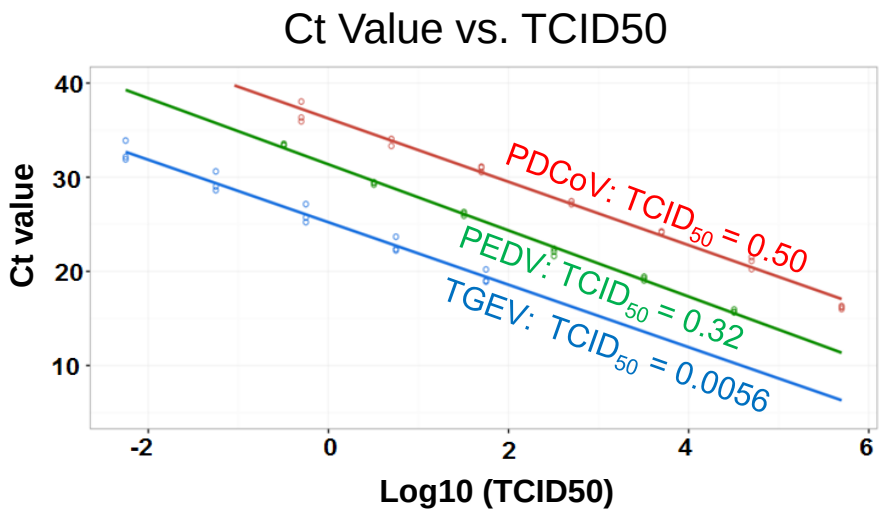
Found the PCR is sensitive to 1-10 viral copies per reaction for each pathogen

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%



Repeatability

Excellent repeatability:

<4% in all cases

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	Interassay	
Limit Of Detection Series	n=3	<4%
Internal Control	n=360	1.7%
PDCoV Pos Control	n=298	1.2%
PEDV Pos Control	n=298	1.1%
TGEV Pos Control	n=298	0.9%

QIAGEN				
	Intra-assay		Inter-assay	
Sample 1	n=6	<1%	n=6	<1%
Sample 2	n=6	<1%	n=6	<1%
Sample 3	n=6	<1%	n=6	<1%
Sample 4	n=6	<1%	n=6	<1%
Pos control	n=6	<1%	n=6	<1%

Analytical Specificity

Confirmed no cross-reactivity with 56 bacterial and viral isolates

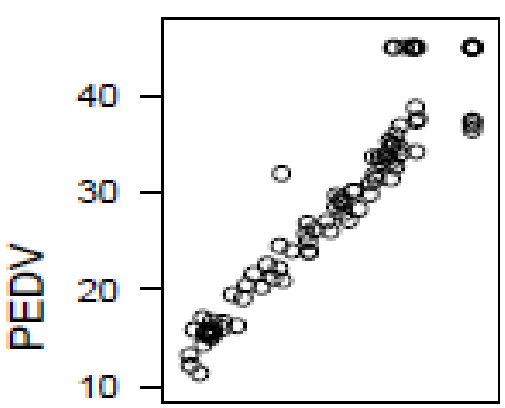
- Porcine cytomegalovirus
- North American Porcine Reproductive and Respiratory Syndrome
- European Porcine Reproductive and Respiratory Syndrome
- Pseudorabies virus
- Swine Influenza virus H1
- Swine Influenza virus H2
- Swine Influenza virus H3
- Porcine Respiratory Corona virus
- Porcine Adenovirus
- 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

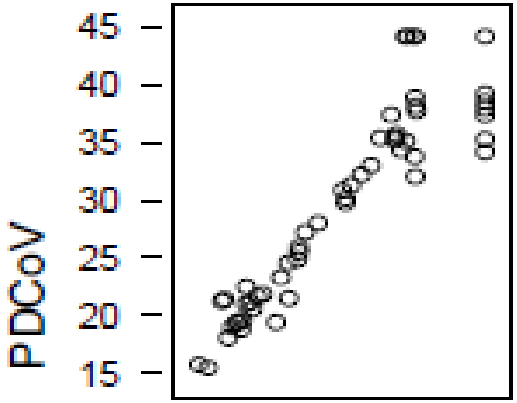
- 360 samples compared between the UMN in-house assays and Qiagen's PDT triplex
  - 127 feces / fecal swabs
  - 99 intestines
  - 92 oral fluids
  - 42 environmental samples

Correlation

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



98%



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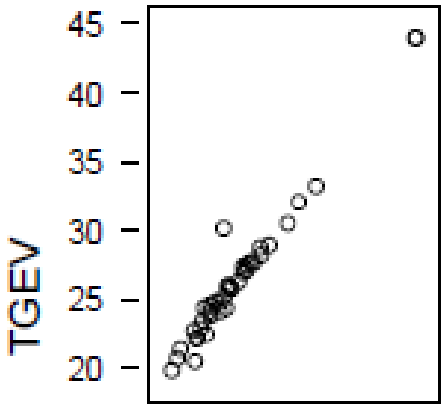

100%

Diagnostic Sensitivity and Specificity

Diagnostic Sensitivity:

(The ability to detect true positives)

PEDV	87%
PDCoV	92%
TGEV	100%

$$\frac{\text{True positives}}{\text{(true positives + false negatives)}}$$

Diagnostic Specificity:

(The ability to detect true negatives)

PEDV	99%
PDCoV	98%
TGEV	100%

$$\frac{\text{True negatives}}{\text{(true negatives + false positives)}}$$

Diagnostic Sensitivity and Specificity

Diagnostic Sensitivity:

(The ability to detect true positives)

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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

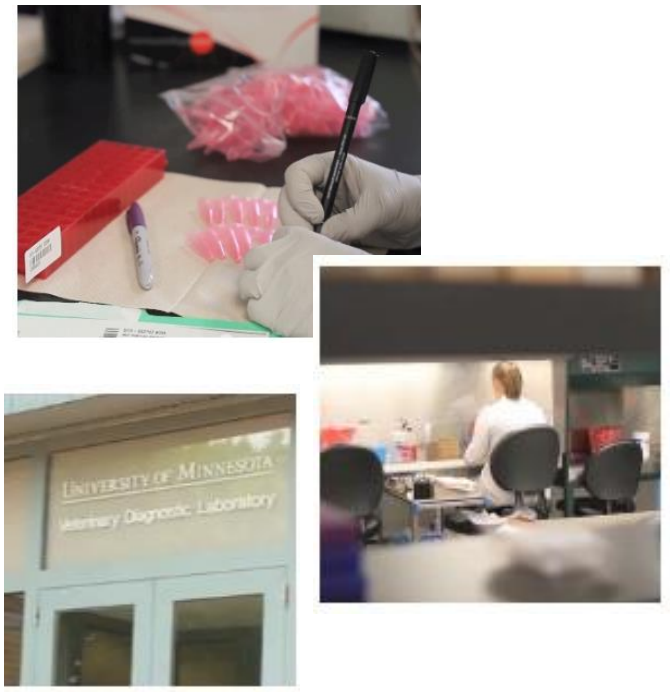
Conclusions

- 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