Development next generation diagnostic platforms for veterinary medicine

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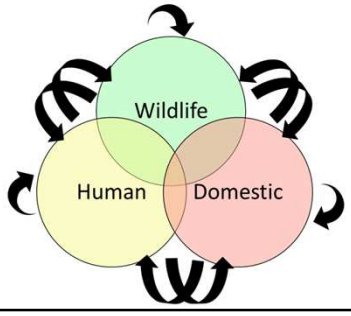
College of Veterinary Medicine
Veterinary Diagnostic Laboratory

Resurgence in the occurrence of infectious diseases…

- Genetic and biological factors
- Environmental factors
- Increasing animal population
- Demography & international travel/commerce

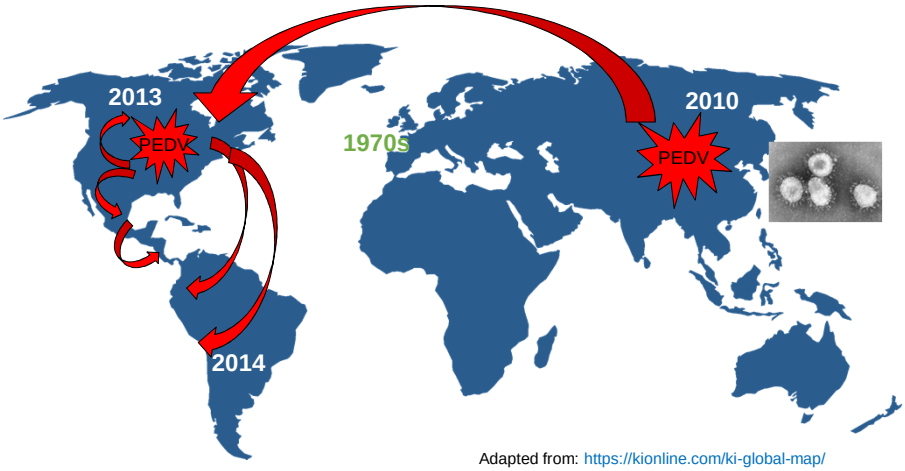


Ability to establish new niches or undergo genetic mutations…

Appearance of new diseases…

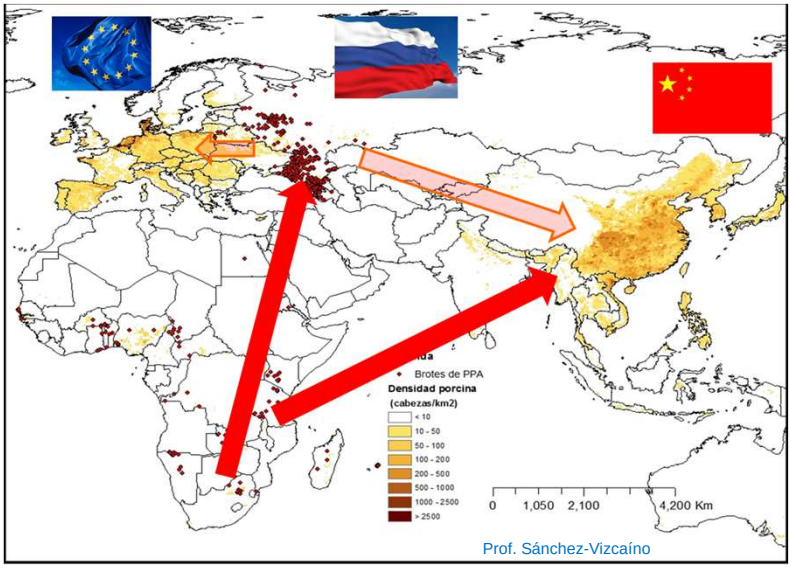
Challenges to → **Diagnostics**
Treatment
Animal health

Porcine epidemic diarrhea virus



Adapted from: https://kionline.com/ki-global-map/

African swine fever virus



Prof. Sánchez-Vizcaíno

Preparedness for EIDs is critical

- Safeguard animal/public health & food supply
- Boost capacity response to emerging diseases
  - Rapid detection-identification-characterization
  - High throughput testing
  - Direct (PCR) & indirect (antibody) methods
  - Applicable to alternative specimens

Alternative specimens for diagnosis

Oral fluids, bulk tank milk…

- Screening large animal populations
- Cost-effective
- User-friendly
- Welfare-friendly

Few kits for few diseases commercially available…

We are in a pressure situation…

- Increasing number of cases every year
- Increasing demand new test new pathogens
- Adaption current/future tests new specimens

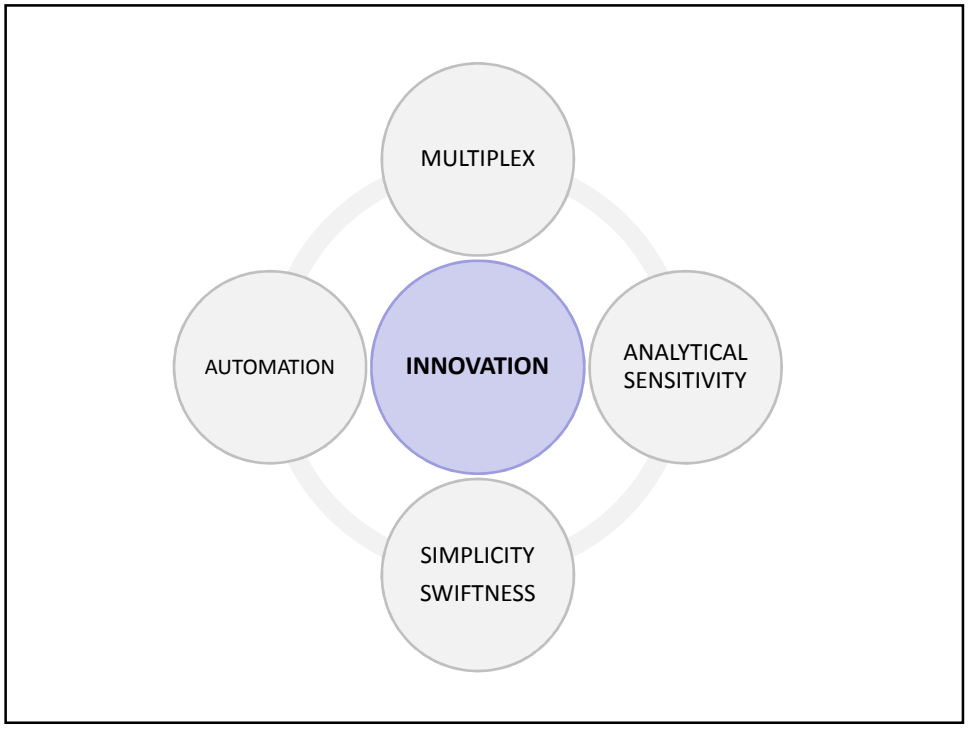
The ongoing challenge for diagnostics…

- Assess current needs and apply contemporary knowledge to:
  - Detection-identification-characterization
  - Further discovery of novel pathogens

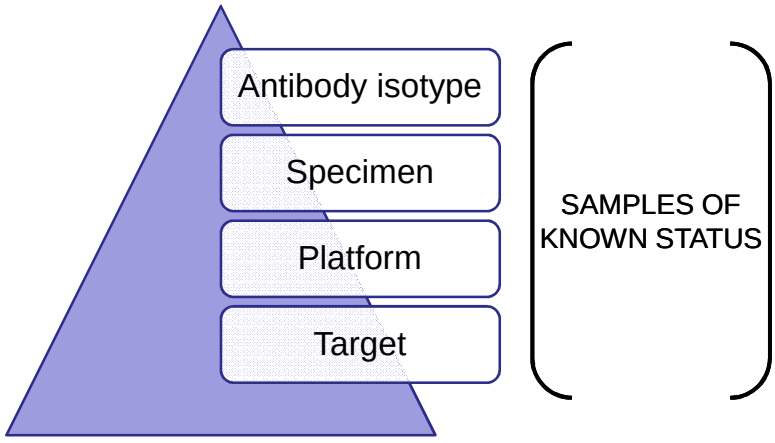
… Faster and better than ever before

Innovation for adaption…

- Emergence of powerful molecular techniques
  - Revealing hidden infections (NGS)
  - Detection dangerous uncultivated agents
  - Detection pathogens which no longer alive
  - Decreasing turnaround time: Timely
  - Minimize human role – Automation

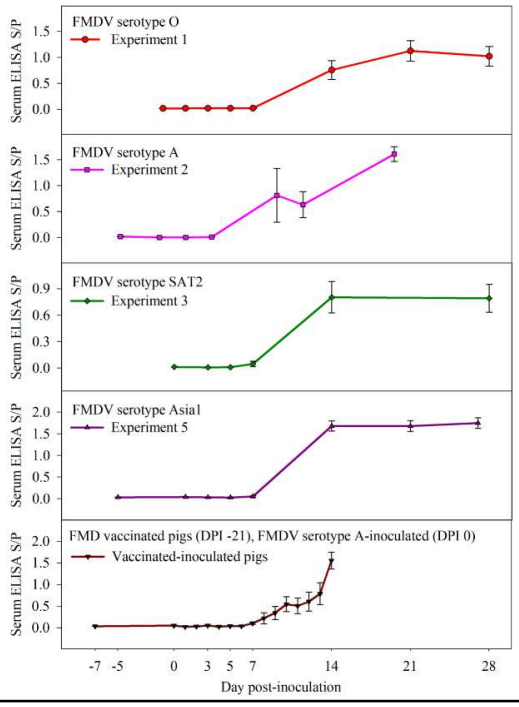


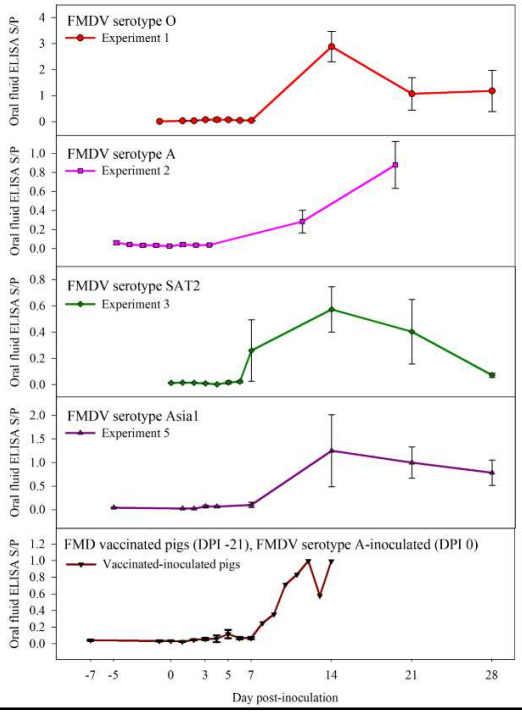
Basics on immunoassay development…



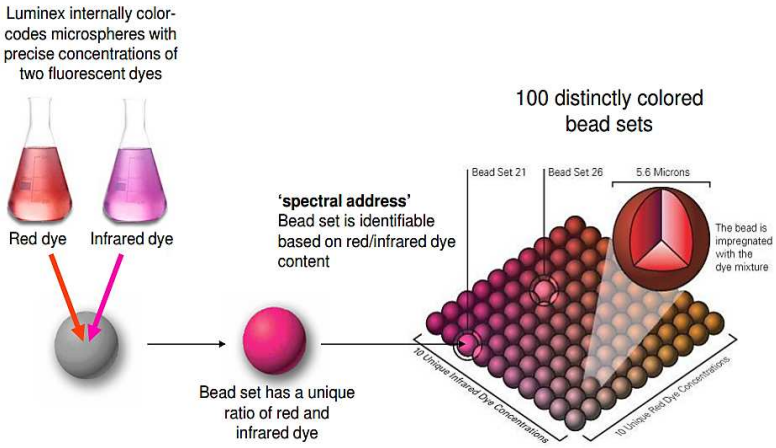
FMDV 3ABC oral fluid/serum iELISA

- Indirect ELISA based on non-structural 3ABC
- Ab detection in swine serum & oral fluids
- Detection of infection different serotypes
- Highly specific (diagnostic/analytical)
- DIVA capabilities
- National Centre for Foreign Animal Disease (NCFAD)



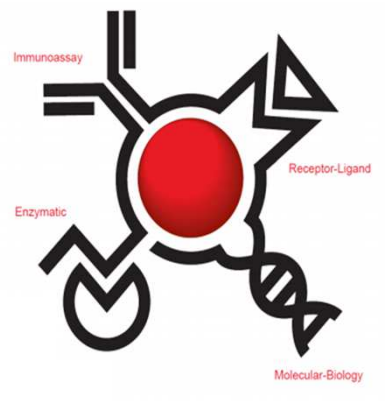


Luminex® multiplex platform

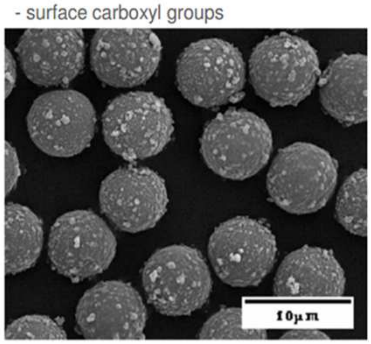


Source: Ocean Ridge Biosciences

Luminex®



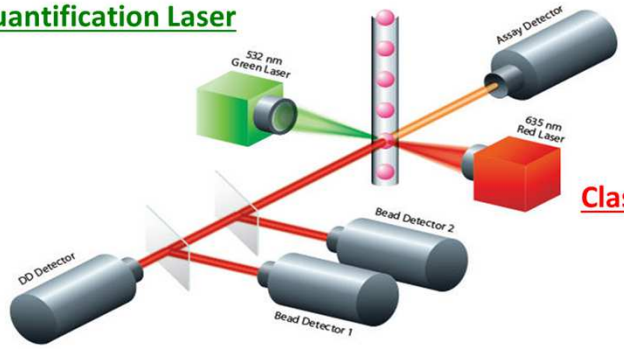
MagPlex®
- Superparamagnetic microspheres
- 6.4 microns
- surface carboxyl groups



Source: Luminex corporation

Luminex®

Quantification Laser



Classification Laser

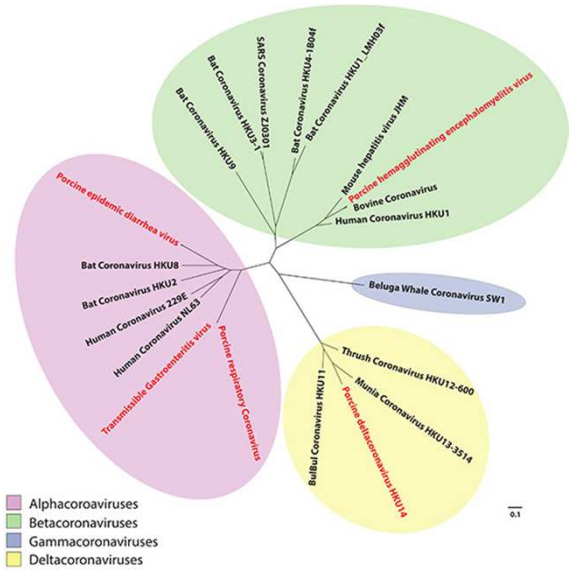
Luminex® can be used as screening and diagnostic platform

- Screening antibody to multiple pathogens
- Screening antibody to multiple antigens
- Enhance analytical sensitivity
- Higher dynamic quantification range
- Lower requirement antigen target & specimen

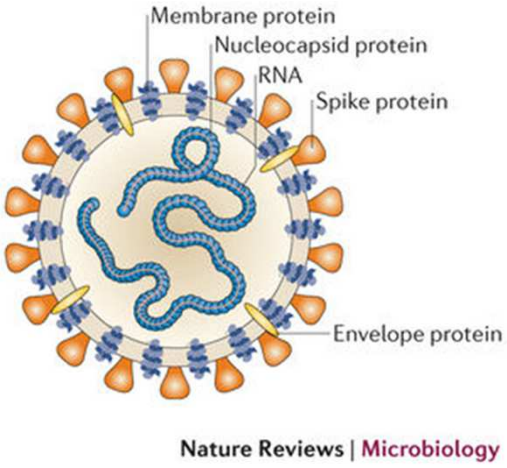
Luminex® can be used as screening and diagnostic platform

- Screening antibody to multiple pathogens
- Screening antibody to multiple antigens
- Enhance analytical sensitivity
- Higher dynamic quantification range
- Lower requirement antigen target & specimen
- Multiplexing technically challenging
- Weak business model (licensing)
- Problem for global distribution
- Expensive (short term) ←→Cheap (long term)

Selection PEDV antigen target

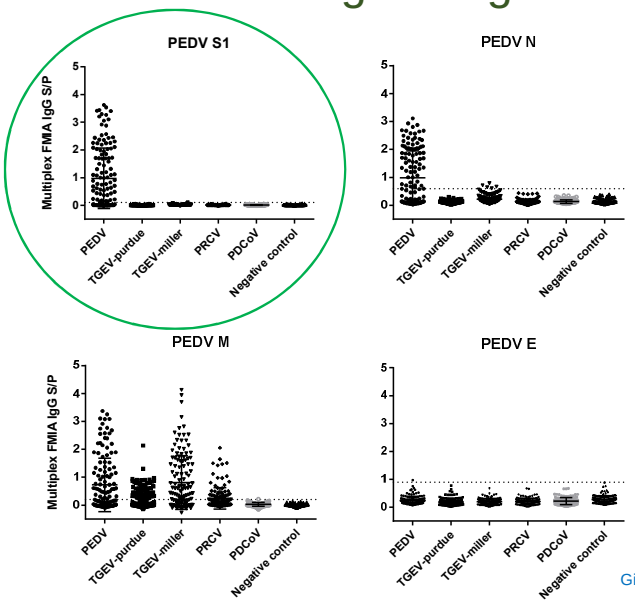


Selection PEDV antigen target



Nature Reviews | Microbiology

Selection PEDV antigen target

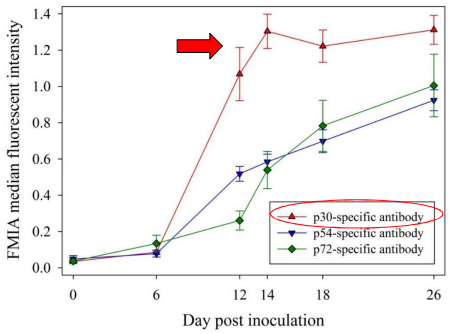


Giménez-Lirola et al. 2017

Selection ASFV antigen target

Which antigen stimulates the best response?

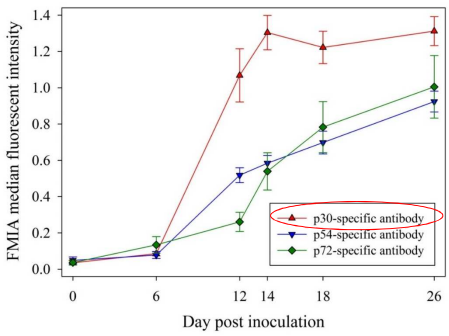
ASFV multiplex Luminex



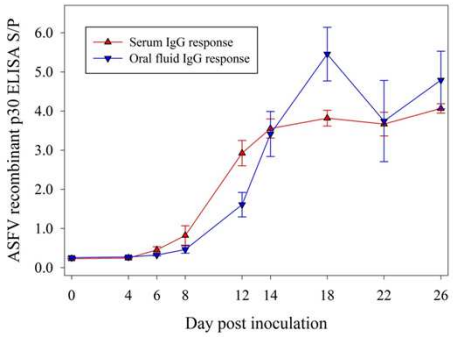
Giménez-Lirola et al. 2016

Selection ASFV antigen target

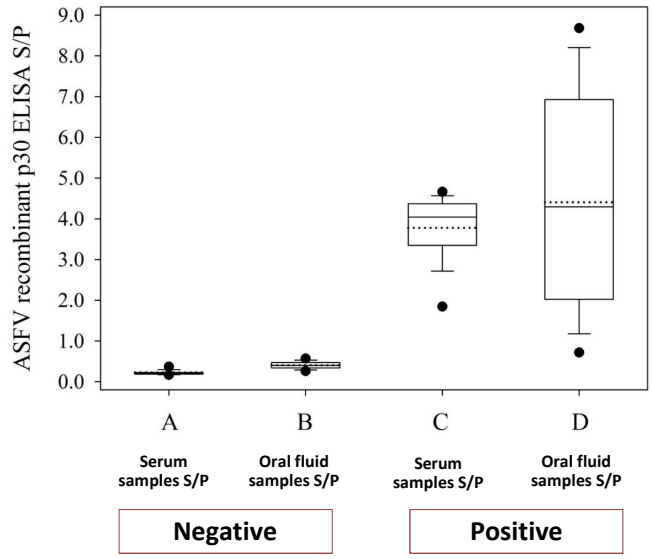
ASFV multiplex Luminex



ASFV p30 dual ELISA



Giménez-Lirola et al. 2016



Research in Veterinary Science 96 (2014) 543–550

Contents lists available at ScienceDirect

Research in Veterinary Science

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Development and validation of a 4-plex antibody assay for simultaneous detection of IgG antibodies against Torque teno sus virus 1 (TTSuV1), TTSuV2, and porcine reproductive and respiratory syndrome virus types 1 and 2

Luis G. Giménez-Lirola [a], Priscilla F. Gerber [a,b], Raymond R. Rowland [c], Patrick G. Halbur [a], Yao-Wei Huang [d,e], Xiang-Jin Meng [d], Tanja Opriessnig [a,f,*]

Journal of Virological Methods 193 (2013) 278–283

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Development of a fluorescent microbead-based immunoassay for the detection of hepatitis E virus IgG antibodies in pigs and comparison to an enzyme-linked immunoassay

Olajide A. Owolodun [a,b], Luis G. Giménez-Lirola [a], Priscilla F. Gerber [a,c].

Vol. 117: 237–243, 2016 | DISEASES OF AQUATIC ORGANISMS | Published January 13

Fluorescent microbead-based im for anti-*Erysipelothrix rhusiopath* detection in cetaceans

Mar Melero [1,*], Luis G. Giménez-Lirola [2], Consuelo José Luis Crespo-Picazo [3], Eva Sierra [4], Daniel Ga Francisco Javier García-Peña [3], Manuel Arbelo [4], Teresa Á José Manuel Sánchez-Vizcaíno [1]

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Simultaneous Detection of Antibodies against Apx Toxins ApxI, ApxII, ApxIII, and ApxIV in Pigs with Known and Unknown *Actinobacillus pleuropneumoniae* Exposure Using a Multiplexing Liquid Array Platform

Luis G. Giménez-Lirola,[a] Yong-Hou Jiang,[a] Dong Sun,[b] Hai Hoang,[b] Kyoung-Jin Yoon,[a] Patrick G. Halbur,[a] Tanja Opriessnig[a,c]

Department of Veterinary Diagnostic and Production Animal Medicine[a] and Department of Veterinary Microbiology and Preventive Medicine,[b] College of Veterinary Me

Journal of Microbiological Methods 91 (2012) 73–79

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Journal of Microbiological Methods

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Development of a novel fluorescent microbead-based immunoassay and comparison with three enzyme-linked immunoassays for detection of anti-*Erysipelothrix* spp. IgG antibodies in pigs with known and unknown exposure

L.G. Giménez-Lirola, C.T. Xiao, P.G. Halbur, T. Opriessnig *

PLOS ONE

RESEARCH ARTICLE

Detection of African Swine Fever Virus Antibodies in Serum and Oral Fluid Specimens Using a Recombinant Protein 30 (p30) Dual Matrix Indirect ELISA

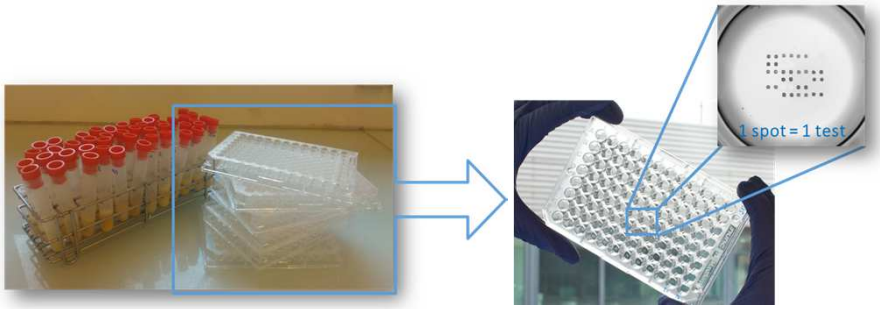
Luis G. Giménez-Lirola[1]*, Lina Mur[2], Belen Rivera[2], Mark Mogler[3], Yaxuan Sun[4], Sergio Lizano[5], Christa Goodell[5], D. L. Hank Harris[5], Raymond R. R. Rowland[6], Carmina Gallardo[7], José Manuel Sánchez-Vizcaíno[2], Jeff Zimmerman[1]

American Society for Microbiology | Journal of Clinical Microbiology®

Reactivity of Porcine Epidemic Diarrhea Virus Structural Proteins to Antibodies against Porcine Enteric Coronaviruses: Diagnostic Implications

Luis Gabriel Gimenez-Lirola,[a] Jianqiang Zhang,[a] Jose Antonio Carrillo-Avila,[b] Qi Chen,[a] Ronaldo Magtoto,[a] Korakrit Poonsuk,[a] David H. Baum,[a] Pablo Piñeyro,[a] Jeffrey Zimmerman[a]

ELISA-like multiplex immunoassay

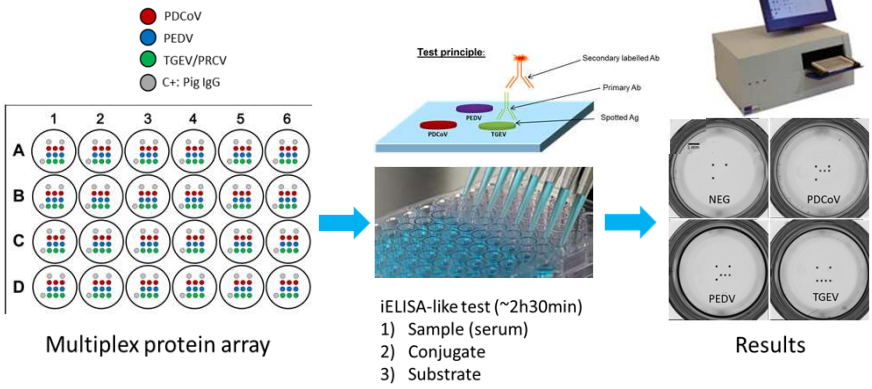


Replacing X 96-wells plates for a single plate: each wells including X tests

- ✓ Increased output/samples
- ✓ Save costs
- ✓ Save time
- ✓ Up to 100 markers

GENES DIFFUSION

AgroDiag PorCoV



Multiplex protein array

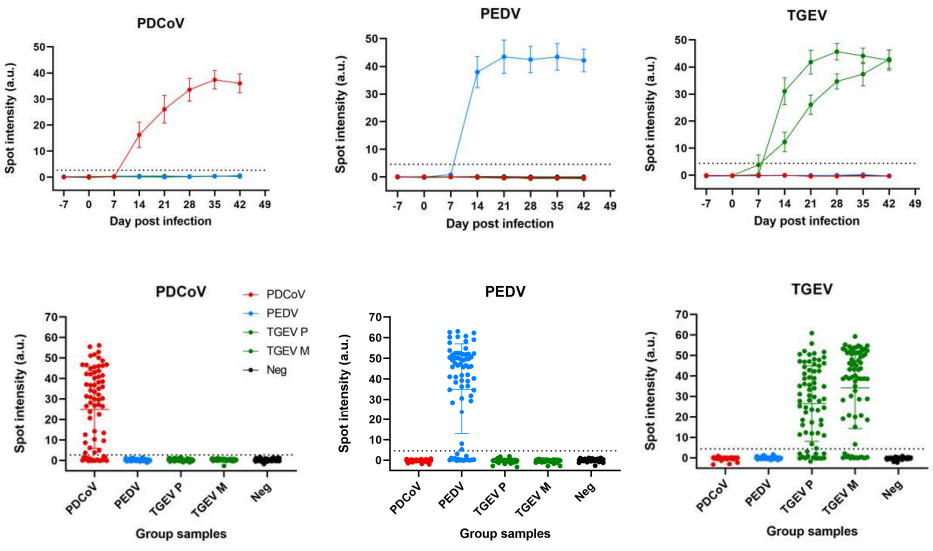
iELISA-like test (~2h30min)
1) Sample (serum)
2) Conjugate
3) Substrate

Results

GENES DIFFUSION

AgroDiag PorCoV

GENES DIFFUSION



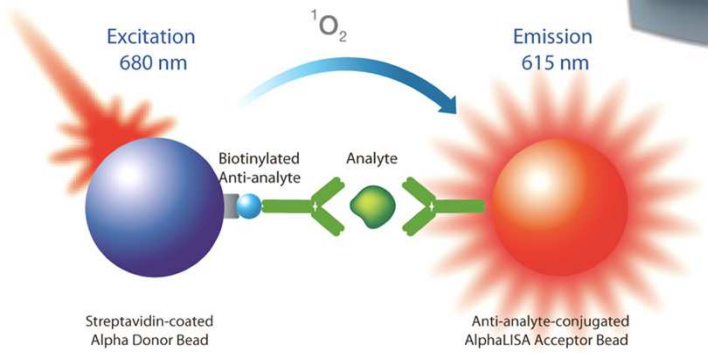
AgroDiag PorCoV

Inoculation group	Cut-off* (normalized intensity)	Number of pigs positives by day post-inoculation								N° samples tested positive**/Total N° positive samples (%)	95% CI
		-7	0	7	14	21	28	35	42		
PDCoV	2.69	0/12	0/12	0/12	7/12	11/12	12/12	12/12	12/12	47/48 (98)	97 to 100
PEDV	4.6	0/12	0/12	1/12	11/12	11/12	11/12	11/12	11/12	44/48 (92)	98 to 100
TGEV Purdue	2.78	0/12	0/12	1/12	9/12	12/12	12/12	11/11	12/12	47/47 (100)	98 to 100
TGEV Miller	4.39	0/12	0/12	0/12	11/12	12/12	12/12	12/12	12/12	48/48 (100)	98 to 100

* Cut-off for **100 % diagnostic specificity**
** Neg : dpi < 0 ; Pos : dpi > 21

GENES DIFFUSION

AlphaLISA platform



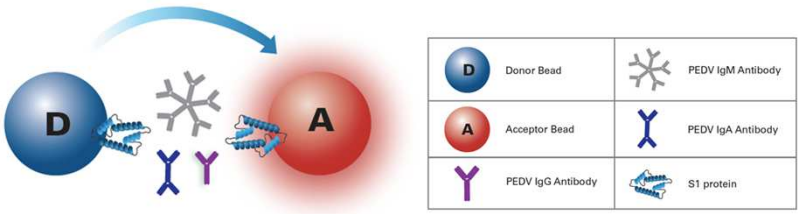
PerkinElmer
For the Better

AlphaLISA platform

- Easy-to-use: no wash assay
- Fast results
- High analytical sensitivity
- Large dynamic range: 4-5 log
- Allow duplex/triplex format
- Specimen-friendly

PerkinElmer
For the Better

PEDV bridge AlphaLISA



- Rapid test (<30min); 1 incubation step; no wash

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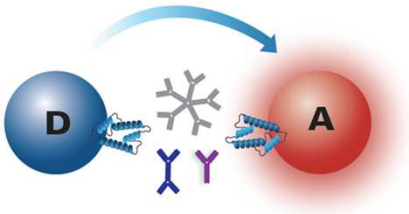
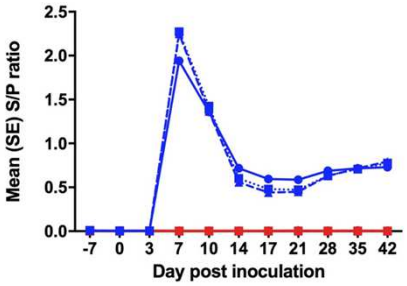
For the Better

PEDV bridge AlphaLISA



D Donor Bead	PEDV IgM Antibody
A Acceptor Bead	PEDV IgA Antibody
PEDV IgG Antibody	S1 protein



- PEDV inoculated (5 minutes)
- PEDV inoculated (30 minutes)
- PEDV inoculated (60 minutes)
- Negative control (5 minutes)
- Negative control (30 minutes)
- Negative control (60 minutes)

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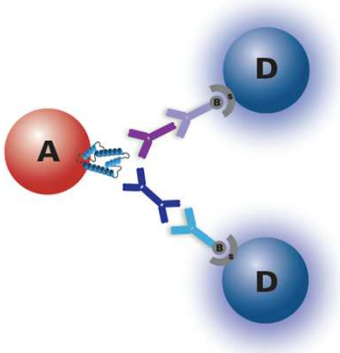
PEDV Ab isotype-specific AlphaLISA



D Donor Bead	PEDV IgA Antibody
A Acceptor Bead	anti-swine IgA (biotinylated)
PEDV IgG Antibody	S1 protein
anti-swine IgG (biotinylated)	Stepatavidin

- 2 hours test; 2 incubation steps; no wash

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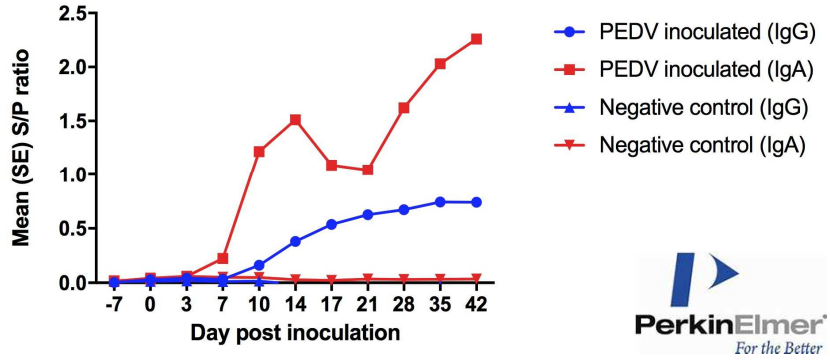
PEDV Ab isotype-specific AlphaLISA

- Evaluation under experimental conditions



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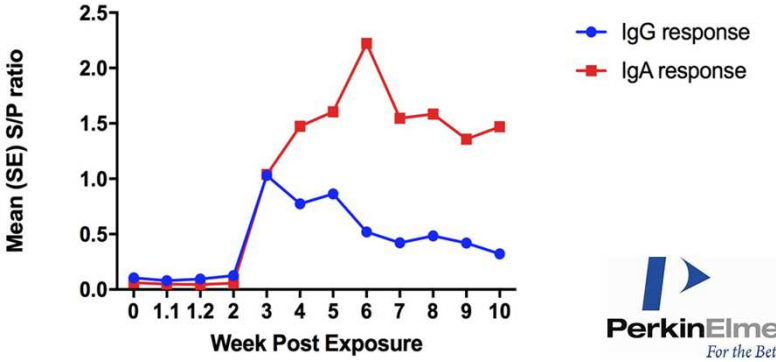
PEDV Ab isotype-specific AlphaLISA

- Evaluation under field conditions



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Improving standard cell-based immunoassays

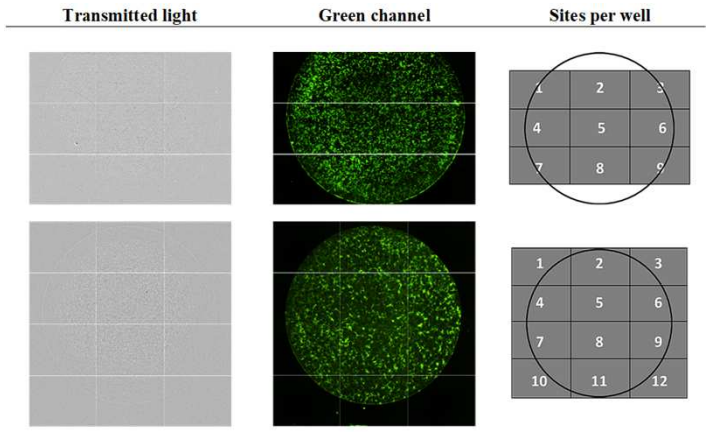
- Balance classic-emerging technologies
- Need address issues related to IFA and FFN
  - Labor intensive
  - Lack standardization
  - Subjective
  - Semi-quantitative results

Converting FFN/IFA into high-throughput testing platforms

SpectraMax MiniMax 300 imaging cytometer

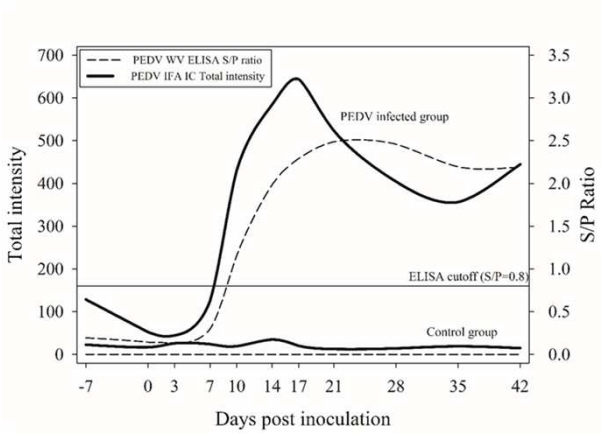
- Imaging
- Cell analysis
- Quantitative results (fluorescent intensity)
- Green/red channels

Imaging and cell analysis



Fluorescent intensity

- Plotting ELISA/IFA data – quantitative results

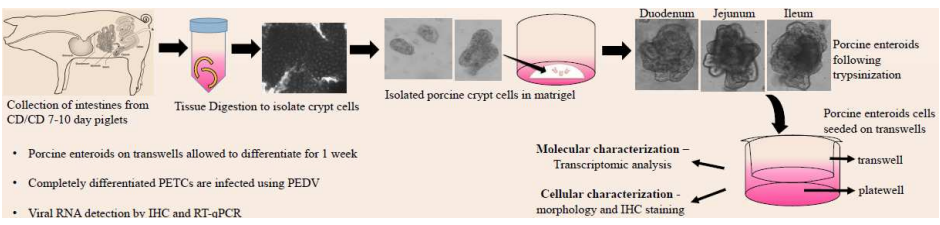


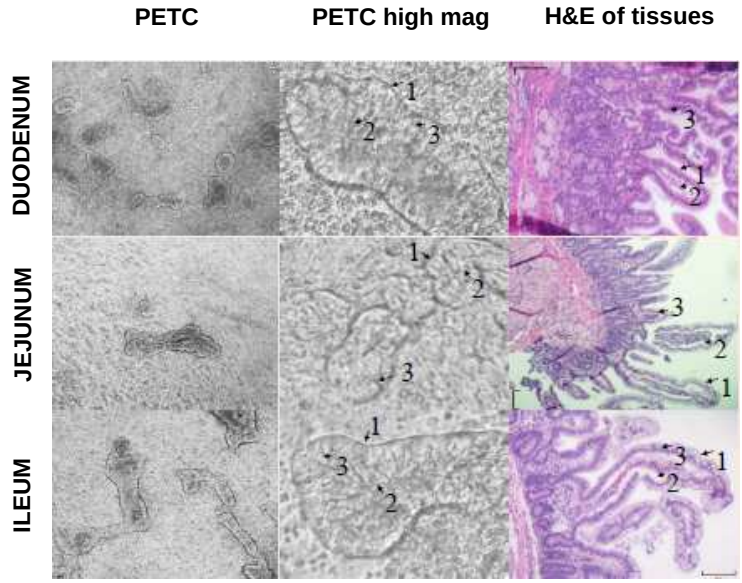
Improvement over classic IFA/FFN

- Reduction time for plate reading (< 3 min)
- Improvement test repeatability/reproducibility
- Improvement precision Ab response estimates
- Currently implemented in ISU-VDL:
  - PEDV IFA
  - PEDV FFN
  - Senecavirus IFA

Porcine enteroids: 3D cell culture model

- Stablish 3D porcine enteroids on transwells
- Culturing crypt cells from small intestine
- *Ex vivo* culture resembling intestinal physiology



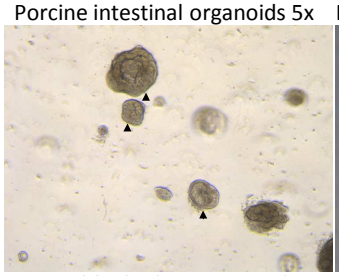


1- Brush border; 2- lamina propria; 3- enterocytes

Porcine enteroids: 3D cell culture model

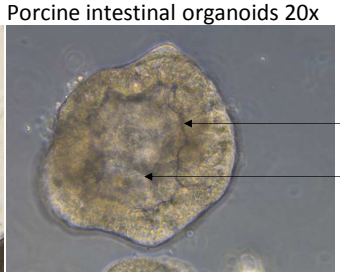
- Images of day 2.

Porcine intestinal organoids 5x



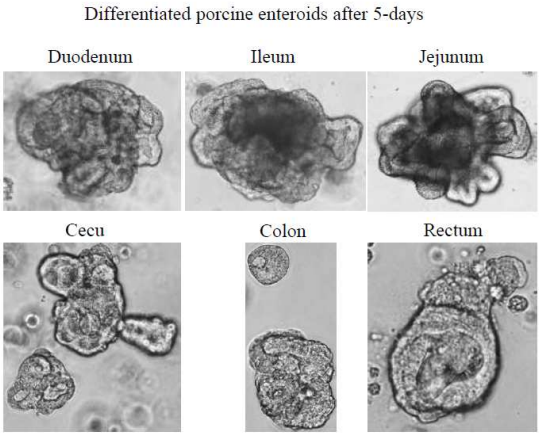
▲ Healthy looking organoids

Porcine intestinal organoids 20x



Porcine enteroids: 3D cell culture model

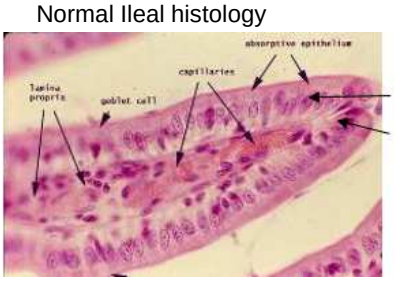
- Images of day 5.



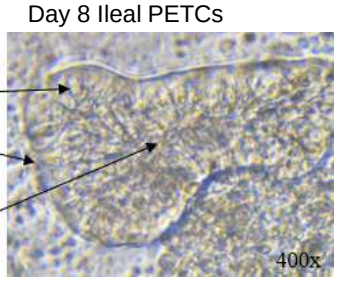
Porcine enteroids: 3D cell culture model

- Images of day 8.

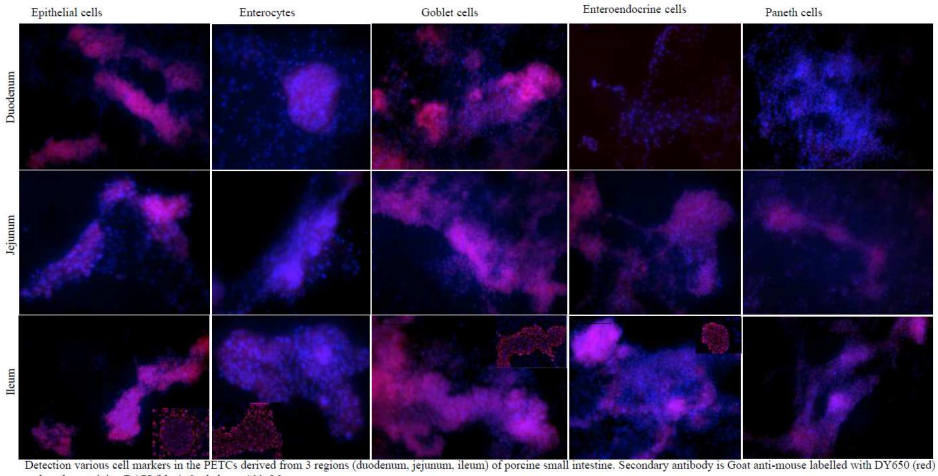
Normal Ileal histology



Day 8 Ileal PETCs



Cell markers in differentiated PETCs

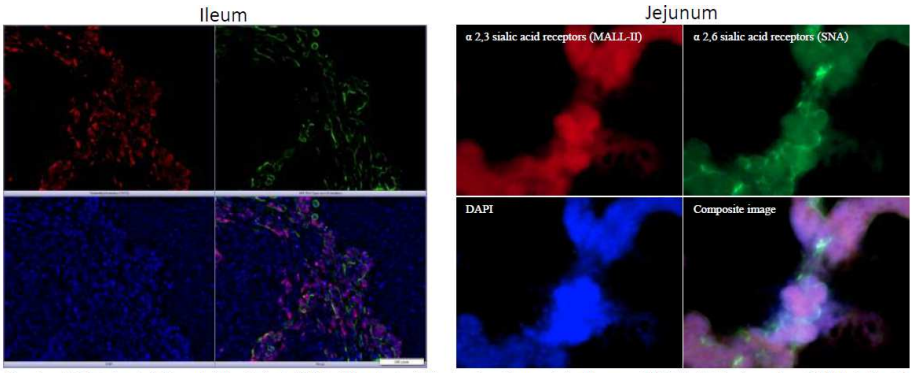


Detection various cell markers in the PETCs derived from 3 regions (duodenum, jejunum, ileum) of porcine small intestine. Secondary antibody is Goat anti-mouse labelled with DY650 (red) and nuclear staining DAPI (blue). Scale bar = 100μM

		Tissue sections			PETCs		
		Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
IHC expression							
Marker	Cell specificity						
Mucin 2	Goblet cells	+	+	+	+	+	+
Villin 1	Enterocytes	+	+	+	+	+	+
Ki-67	Proliferation	+	+	+	+	+	+
Occludin	Tight Junction protein	+	+	+	+	+	+
Lysozyme C	Paneth	+ weak	+	+	+	+	+
Chromogranin A	Enteroendocrine	+ weak	+	+	+	+	+
Pan cytokeratin	Epithelial cells	+	+	+	+	+	+
Lectin expression	α2,3 & α2,6 sialic acids	+	+	+	+	+	+
Gene expression							
Marker	Cell specificity						
PCNA	Proliferation	+	+	+	+	+	+
MUC2	Goblet cells	+	+	+	+	+	+
CHGA	Enteroendocrine	+	+	+	+	+	+
SLC5A1/SGLT1	Mature enterocytes	+	+	+	+	+	+
LYZ	Paneth	+	+	+	+	+	+
FZD5	Paneth	+	+	+	+	+	+
EPHB2	Paneth	+	+	+	+	+	+
LGR5	Crypt cells	+	+	+	+	+	+

Summary of various cell marker expression (fluorescence immunohistochemistry and gene expression) in the PETCs derived from 3 regions (duodenum, jejunum, ileum) of porcine small intestine.

Small intestinal PETCs lectins expression

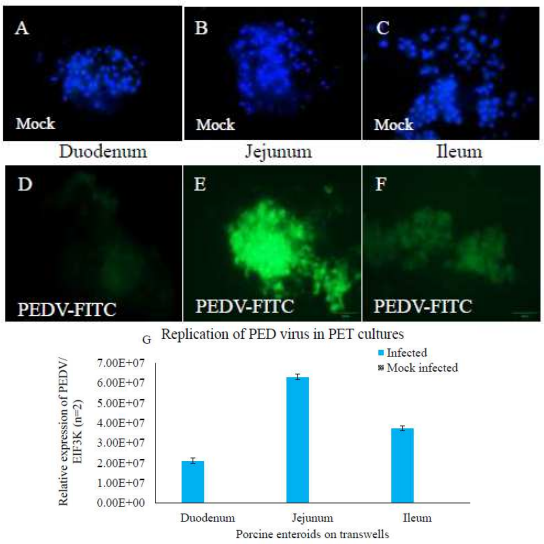


Detection of α2,3 (red) and α2,6 (green) sialic acids in the PETCs of Ileum by lectin histochemistry. Fluorescein isothiocyanate (FITC) labelled *Sambucus nigra (*SNA) lectin for α2,6 and biotinylated *Maackia amurensis (*MAL) II lectin + streptavidin conjugate labelled with Dy650 for α2,3 SA receptors. Nuclear staining DAPI (blue). Scale bar = 100μM

Multiple applications of enteroids…

- Intestinal physiology and nutrition
- Screening drugs
- Immunopathology
- Innate immune response
- Pathogen – microbiota interaction
- Virus isolation and propagation
- Bioassay

Susceptibility small intestinal PETCs to PEDV infection



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GENES DIFFUSION

IOWA STATE UNIVERSITY
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Veterinary Diagnostic Laboratory

Thank you !

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