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Diagnostics of Coronavirus infections in humans and animals

Carsten Schroeder, DVM, PhD, INDICAL BIOSCIENCE
Douglas Marthaler, PhD, INDICAL Inc.

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Diagnostics of Coronavirus infections in humans and animals

- Introduction to Coronaviruses
- Coronaviruses in
 - Humans
 - Companion animals
 - Livestock
 - Poultry
- Examples for Coronavirus testing solutions
- Summary and conclusions



Douglas Marthaler,
PhD

Molecular Biologist

Science and Technology
Sales Manager for North
America.

douglas.marthaler@indical.com



Carsten Schroeder,
DVM, PhD

Veterinarian

Director Business Area
Veterinary Diagnostics
for Reference Laboratories,
Dairy and Aquaculture.

carsten.schroeder@indical.com



With over two decades of experience,
we are a leader in molecular diagnostics
for vet-specific applications

Coronavirus diversity

Human

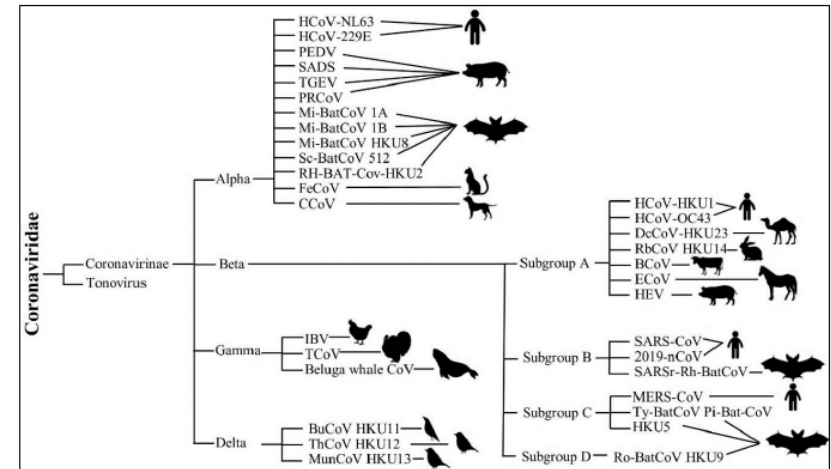
- 229E
- NL63
- OC43
- HKU1
- Severe Acute Respiratory Syndrome (SARS-CoV)
- Middle East Respiratory Syndrome (MERS-CoV)
- 2019-novel coronavirus (SARS-CoV-2)

Livestock and poultry

- Porcine Epidemic Diarrhea Virus (PEDV)
- Transmissible Gastroenteritis Virus (TGEV)
- Porcine Respiratory Coronavirus (PRCoV)
- Swine Enteric Alphacoronavirus (SEACoV)
- Bovine Coronavirus (BCoV)
- Infectious Bronchitis Virus (IBV)

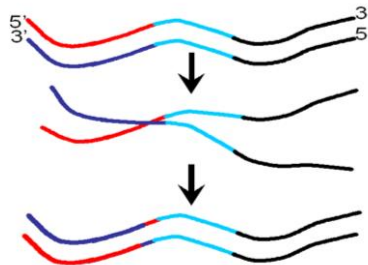
Companion Animals

- Feline Coronavirus (FCoV)
- Canine Enteric Coronavirus (CECoV)
- Canine Respiratory Coronavirus (CRCoV)
- Equine Coronavirus (ECoV)

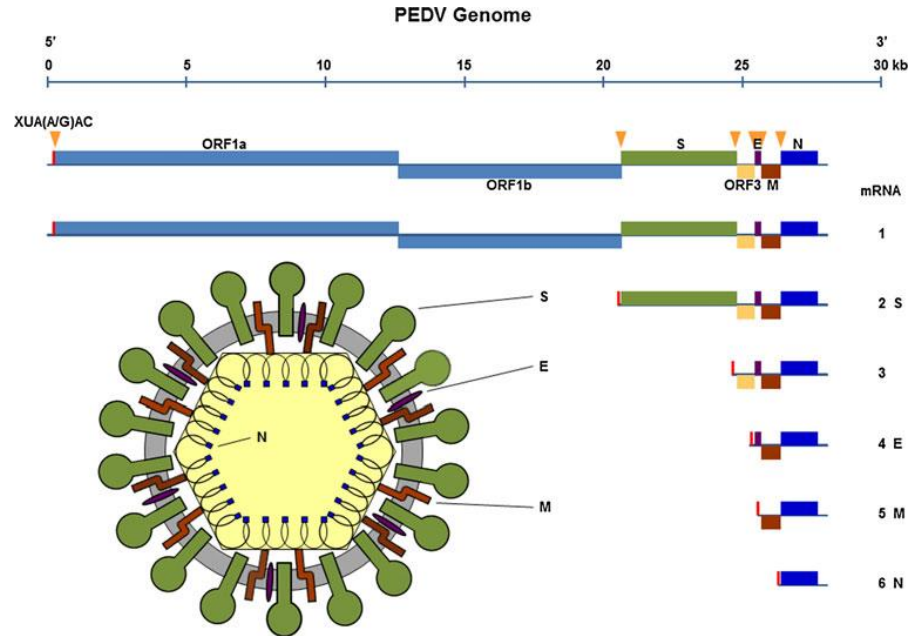


Coronavirus genome

- Envelope, positive sense, single-stranded RNA virus
- Viral genomes of 26–32kb
- Recombination events generate new strains, which can have altered tissue or host tropism
 - Humans
 - Domesticated and wild mammalian and avian species



Mechanism of recombination on nucleic acid strands



Song D, Park B. *Virus Genes*. 2012 Apr;44(2):167-75. doi: 10.1007/s11262-012-0713-1. Epub 2012 Jan 22. Review. PubMed PMID: 22270324.

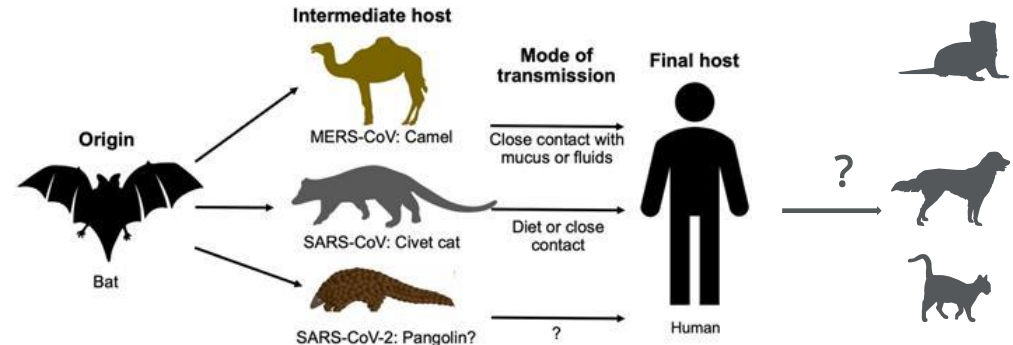
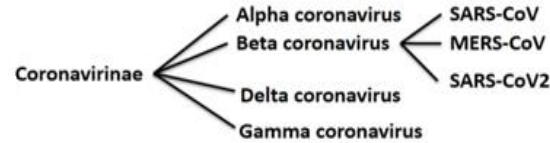
Common Coronaviruses in humans

- Four common Coronaviruses
 - **229E** (α -Coronavirus)
 - **NL63** (α -Coronavirus)
 - **OC43** (β -Coronavirus)
 - **KHU1** (β -Coronavirus)
- First human Coronaviruses identified in the mid-1960s
- Mild symptoms and associated as the common cold
- Reinfections even with the same strain
 - Lack of sterilizing immunity (immunity wanes over time)



β -Coronaviruses

- Severe Acute Respiratory Syndrome, SARS-CoV
- Middle East Respiratory Syndrome, MERS-CoV
- 2019 novel Coronavirus, SARS-CoV-2



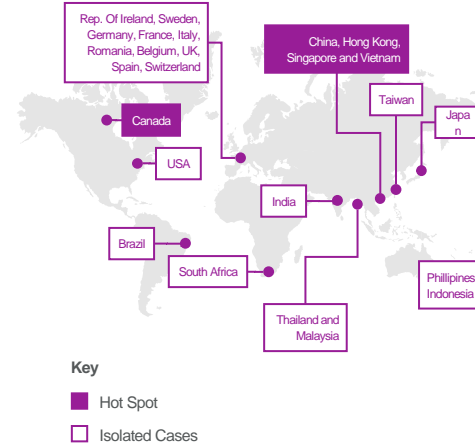
Modified from: Yi Y, Lagniton PNP, Ye S, Li E, Xu RH. COVID-19: what has been learned and to be learned about the novel coronavirus disease. Int J Biol Sci 2020; 16(10):1753-1766. doi:10.7150/ijbs.45134. Available from <http://www.ijbs.com/v16p1753.htm>

SARS-CoV

- Discovered in Guangdong, China in November 2002
- Spread to several countries in North and South America, Europe, and Asia
- 8,096 cases, 774 deaths
- Severity of disease varied based on respiratory co-infections, dose and route of infections, and pre-underlining conditions
- No new cases since 2004



(Stavrinos and Guttman, 2004) www.who.int/csr/sars/diagnostictests/en/



Virus detection

Specimens:

Nasal/pharyngeal swabs, respiratory secretions, blood, stool and tissues

Method: RT-qPCR

Antibody detection

Specimen:

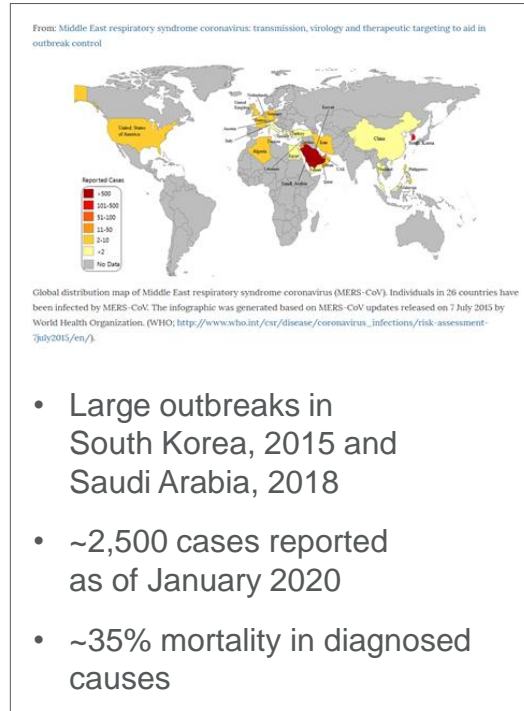
Serum (paired serum samples)

Methods:

IgM and/or IgG by ELISA and Immunofluorescent Antibodies (IFA)

MERS-CoV

- Discovered in Saudi Arabia, 2012
- Symptoms range from asymptomatic to severe
- Fever, cough, diarrhea, and shortness of breath
- Severe symptoms typical associated with other underlying health conditions




Virus detection

Specimens:

Nasopharyngeal/oropharyngeal swabs, sputum, and lower respiratory tract, nasopharyngeal and nasal aspirates

Methods: RT-qPCR, virus isolation (special labs only)



Antibody detection

Specimen:

Serum (paired serum samples)

Methods: IgA, IgG, and/or IgG ELISA

SARS-CoV-2

- Discovered in Wuhan, China, December 2019
- Pandemic
- Symptoms from asymptomatic to severe
 - Common cold like or influenza symptoms
 - Severe symptoms typical associated with other underlying health conditions
- Transmits via exhaled droplets, aerosols, and contaminated surfaces



Virus detection
Specimens:

Nasopharyngeal/oropharyngeal swabs, sputum, and lower respiratory tract, nasopharyngeal and nasal aspirates

Methods: RT-qPCR, virus isolation (special labs only)


Antibody detection
Specimen:

Serum (paired serum samples)

Methods: IgA, IgG, and/or IgG ELISA

SARS-CoV-2

Prototype Coronavirus genome



Summary table of available protocols in this document

Institute	Gene targets
China CDC, China	ORF1ab and N
Institut Pasteur, Paris, France	Two targets in RdRP
US CDC, USA	Three targets in N gene
National Institute of Infectious Diseases, Japan	Pancorona and multiple targets, Spike protein
Charité, Germany	RdRP, E, N
HKU, Hong Kong SAR	ORF1b-nsp14, N
National Institute of Health, Thailand	N

www.who.int/docs/default-source/coronaviruse/whoinhouseassays.pdf?sfvrsn=de3a76aa_2

 RT-qPCR

- Variety of PCR protocols with different gene targets
 - Pan-Corona
 - SARS related Coronaviruses
 - SARS-CoV-2

Coronaviruses in companion animals

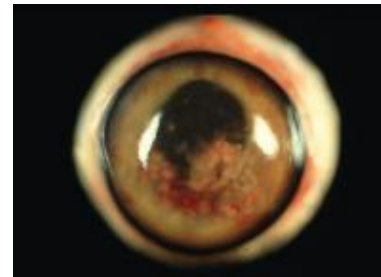




Feline coronavirus (FCoV), α -Coronavirus

Two clinical forms:

- Feline enteric coronavirus (FECV)
 - Infects the intestine
 - 5-10% of infected cats
- Feline infectious peritonitis virus (FIPV)
 - Systemic infections
 - Two major forms
 - Wet: fluid in the cat abdomen
 - show progressive, nonpainful abdominal distension and death within weeks to months
 - Dry: small accumulations of inflammatory cells, granulomas, in various organs
 - Clinical signs depend on which organ is affected



Virus detection

Specimens: Pleural or abdominal effusion, swabs, blood, feces, and lymph node

Methods: RT-qPCR, Immunofluorescences Antibody (IFA), Immunostaining

Antibody detection

Specimens: Serum and ascites

Methods: FCoV antibody ELISA, IFAT, and Agar Gel Immunodiffusion (AGID)



Canine Enteric Coronavirus (CECoV), α -Coronavirus


- Discovered in 1971
- Intestinal infection in dogs, especially puppies
- Causes abdominal discomfort and diarrhea for a few days
- Subclinical infections occur
- High seroprevalence



Virus detection

Specimen: Feces

Methods: RT-qPCR, IFAT (FITC conjugated antibodies) Lateral Flow devices (LFDs), and electron microscopy



Antibody detection

Specimen: Serum

Methods: ELISA and Immunofluorescences Antibody





Canine Respiratory Coronavirus (CRCoV), β -Coronavirus

- Discovered in 2003
- High morbidity, low mortality
- Dry, hacking cough
- Mild and self-limiting
- Associated with canine infectious respiratory disease (CIRD)
 - *Bordetella bronchiseptica*, canine parainfluenza, canine adenovirus type 1 and 2, canine herpesvirus, *Mycoplasma species*, canine pneumovirus, and influenza viruses

Virus detection

Specimen:

Pharyngeal swab

Method: RT-qPCR

Antibody detection

Specimen: Serum

Methods: ELISA and Immunofluorescences
Antibody

Coronaviruses in horses





Equine Coronavirus (ECoV), β -Coronavirus

- Discovered in 1999
- Asymptomatic infections can occur
- Diarrhea and colic in adult horses
- May cause fever and respiratory disease
- Rapid progression can lead to death
- Most cases resolved with supportive care



Virus detection

Specimen: Feces

Methods: RT-qPCR, electron microscopy, VI



Antibody detection

Specimen: Serum

Method: ELISA (S1 protein)

Coronaviruses in cattle





Bovine Coronavirus (BCoV), β -Coronavirus

Distinct clinical syndromes in cattle

Calf Diarrhea and Calf Respiratory BCoV Infections

- One to 4-week-old calves
- Severe, malabsorptive diarrhea, dehydration and often death
- Concurrent fecal and nasal shedding
- Often combined with Rotavirus, E. coli, and Cryptosporidium

Winter Dysentery BCoV Infections

- Adult cattle during the winter months
- Characterized by hemorrhagic diarrhea, frequent respiratory signs
- Reduction in milk production in dairy cattle

Shipping Fever BCoV Infections

- Respiratory disease in feedlot cattle
- Alone or in combination with several viruses (BRSV, Parainfluenza-3 virus, BHV-1, and BVDV)

Virus detection

Specimens: Trachea and tracheal swabs, cecal tonsils, and cloacal swabs

Methods: RT-qPCR, electron microscopy, virus isolation, Lateral Flow Devices

Antibody detection

Specimen: Serum

Methods: ELISA and Hemagglutination Inhibition (HI)

Coronaviruses in swine



The three main swine enteric coronaviruses

Porcine Epidemic Diarrhea Virus (PEDV), α -Coronavirus

- Discovered in Europe, 1970s
- Report in Asia, 1990s
- Introduced into North and South America in 2013
 - Killed >7M pigs within a year
- Rereported in Europe in 2014
 - Less severe outbreaks
- Mortality up to 100% in preweaned piglets
 - Newborn piglets die within 5 days

Transmissible Gastroenteritis Virus (TGEV), α -Coronavirus

- Discovered in 1946
- In piglets less than 1 week old, the mortality rate is close to 100%.
- Finishing pigs can be reservoir
- Rare cases in Europe and the U.S.

Porcine deltacoronavirus (PDCoV), δ -Coronavirus

- Discovered in Hong Kong, 2014
 - Study to identify new coronaviruses in animals
- Retrospective study identified in 2009
- Identified in US, 2014
- Lower mortality rates compared to PEDV and TGEV
- Viral origins believed to be an avian coronavirus

The three main swine enteric coronaviruses – identifying PEDV, TGEV and PDCoV

Virus detection

Specimens: Fecal, fecal swabs, gut tissue, oral fluids, environmental samples

Methods: RT-qPCR , mostly Triplex PCR for combined testing
(e.g. virotype PEDV/TGEV/PDCoV RT-qPCR)

Antibody detection

Specimen: Serum

Methods: ELISA, Immunofluorescence Antibody (IFA)



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Reagents for research use only, not for use in diagnostic procedures. Regulatory requirements vary by country, products may not be available in your geographic area.
PCR and ELISA kits availability/distribution: Outside the U.S. and Canada



Porcine Respiratory Coronavirus (PRCoV), α -Coronavirus

- Discovered in 1986
- Spike gene deletion of TGEV
- Cell tropism changed to respiratory
- Low mortality and morbidity
- Subclinical or mild respiratory symptoms with other respiratory pathogens
- Generally, enzootic in swine herd
- Antibodies demonstrate cross protection against TGEV



Virus detection

Specimens: Nasal swabs, lung tissue, and oral fluids

Methods: RT-qPCR, electron microscopy, and VI



Antibody detection

Specimen: Serum

Methods: ELISA, IFAT



Swine enteric alphacoronavirus (SeACoV), α -Coronavirus

- Discovered in China, 2017
- Retrospective study identified in August 2016
- Limited to Guangdong and Fujian, China
- Acute vomiting and watery diarrhea
- Pathogenicity of the virus is controversial
- Mild-moderate diarrhea or subclinical infections



Virus detection

Specimens:

Feces and intestinal tissues

Methods: RT-qPCR , Virus Isolation (VI)



Antibody detection

Specimen: Serum

Methods: ELISA and Immunofluorescences Antibody (IFA)



Porcine hemagglutinating encephalomyelitis virus (PHEV), β -Coronavirus

- Discovered in Canada, 1957
- Causes vomiting and wasting disease (VWD) and/or encephalomyelitis
- Replication in respiratory tract and spread to the central nervous system
- Morbidity and mortality is age-dependent
- Generally reported only in piglets > 4 weeks old
- Highly prevalent and circulates subclinically in most swine herds worldwide.
- Clinical significance remains uncertain in most of the swine-producing countries



Virus detection

Specimens: Brain or lung tissues

Methods: RT-qPCR, Electron Microscopy, Virus Isolation



Antibody detection

Specimen: Serum

Methods: Immunofluorescence antibody (IFA), Hemagglutination Inhibition

Coronaviruses in chicken





Infectious Bronchitis Virus (IBV), γ -Coronavirus

- Discovered in 1931
- Highly contagious respiratory disease
- Present world wide
- Infects respiratory tract, gut, kidney and reproductive systems
- High mortality in young chickens
- Coughing, and nasal discharge
- Decreased egg production, misshapen and discoloration of eggs
- High incidence of mutations leads to large number of strain specific vaccines



Virus detection

Specimen: trachea and tracheal swabs, cecal tonsils, and cloacal swabs

Method: RT-qPCR, strain typing, virus isolation



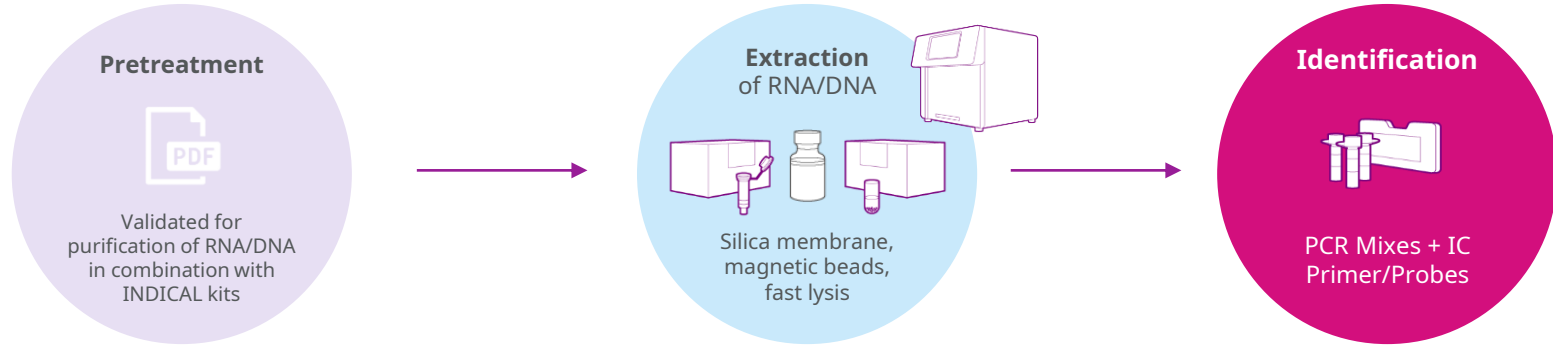
Antibody detection

Specimens: Serum

Method: ELISA, virus neutralisation test, Agar Gel Immunodiffusion (AGID), Hemmagglutination Inhibition (HI)

Monitoring vaccine response and flock performance based on ELISA titers

Molecular Testing Workflows



Pretreatment S1

- Tracheal-, oropharyngeal-, blood- swabs

Pretreatment T1

- Tissue

Pretreatment F1

- Fecal samples

Pretreatment C1

- Samples on FTA cards

Extraction of RNA/DNA

- IndiSpin Pathogen Kit
(Old name: QIAamp cador Pathogen Mini Kit)
- IndiSpin QIAcube HT Pathogen Kit
(Old name: cador Pathogen 96 QIAcube HT Kit)
- IndiMag Pathogen Kit
(Old name: MagAttract 96 cador Pathogen Kit)
- virotype Tissue Lysis Reagent
(Fast lysis buffer)

Identification

- PCR-Mixes*
 - virotype Mix +IC (TAMRA)-RNA
 - virotype Mix +IC (JOE)-RNA
 - virotype Mix (for use with intype IC)
- Primers/Probes*
 - virotype SARS-CoV (E Sarbeco)
 - virotype SARS-CoV-2 (RdRP)
 - virotype SARS-CoV-2 (IP4)
 - virotype PEDV/TGEV/PDCoV

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Coronavirus – Summary and conclusions

- Four genus of Coronavirus: α , β , γ , and δ
- Cause mild to severe enteric, respiratory, or systemic disease
- Recombination contributes to new strains with altered tissue or host tropisms
- Zoonosis and zoonothroponosis
- Multiple new Coronaviruses
 - 3 new CoVs identified in humans
 - SARS-CoV, MERS-CoV, and SARS-CoV-2
 - 3 new CoVs identified in domesticated mammals
 - Swine Enteric Alphacoronavirus, Porcine Deltacoronavirus, and Canine Respiratory Coronavirus
- Diagnostics mostly by RT-qPCR
 - Sample extraction and RT-qPCR solutions from INDICAL

Thank you !

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douglas.marthaler@indical.com

www.indical.com

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INDIKANG BIOTECH, China

ella.wang@indikang.com

