Libyan International Medical University
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Second Year Block VII

# A Novel Coronavirus from Patients With Pneumonia in China, 2019

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# Emerging and reemerging pathogens are global challenges for public health.

Coronaviruses are enveloped RNA viruses that are distributed broadly among humans, other mammals, and birds and that cause respiratory, enteric, hepatic, and neurologic diseases.

Six coronavirus species are known to cause human disease.

Four viruses

- 229E
- OC43
- NL63
- HKU1

are prevalent and typically cause common cold symptoms in immunocompetent individuals.

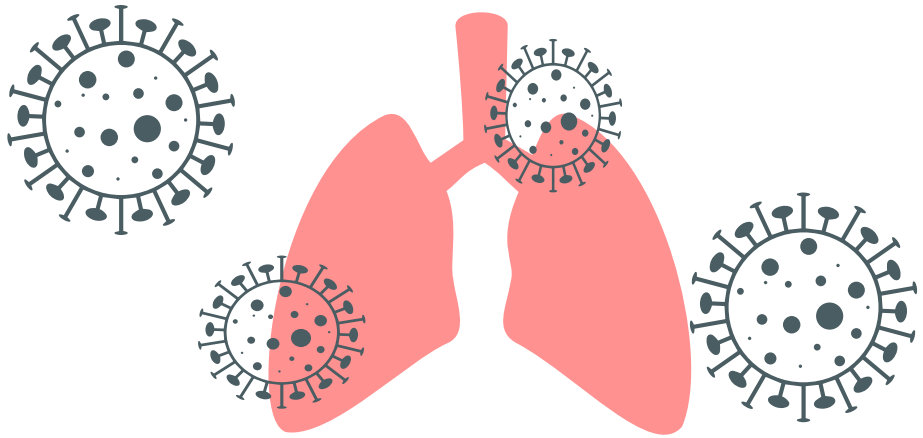
# The two other strains

- severe acute respiratory syndrome coronavirus (SARS-CoV) was the causal agent of the severe acute respiratory syndrome outbreaks in 2002 and 2003 in Guangdong Province, China.
- Middle East respiratory syndrome coronavirus (MERS-CoV) was the pathogen responsible for severe respiratory disease outbreaks in 2012 in the Middle East.

Given the high prevalence and wide distribution of coronaviruses, the large genetic diversity and frequent recombination of their genomes, and increasing human–animal interface activities, novel coronaviruses are likely to emerge periodically in humans owing to frequent cross-species infections and occasional spillover events.

## In late December 2019

Several local health facilities reported clusters of patients with pneumonia of unknown cause that were epidemiologically linked to a seafood and wet animal wholesale market in Wuhan, Hubei Province, China.



## On December 31, 2019,

The Chinese Center for Disease Control and Prevention (China CDC) dispatched a rapid response team to accompany Hubei provincial and Wuhan city health authorities and to conduct an epidemiologic and etiologic investigation.

# Methods

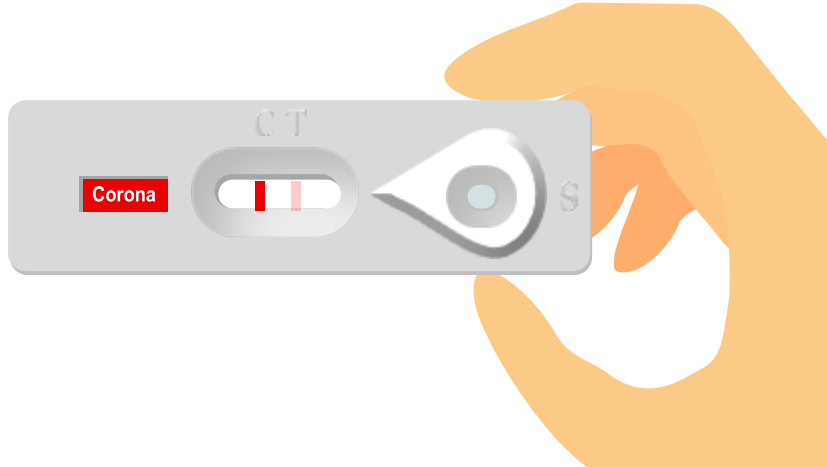
- Viral Diagnostic Methods
- Isolation of Virus
- Transmission Electron Microscopy
- Viral Genome Sequencing

# Viral Diagnostic Methods

Four lower respiratory tract samples, including bronchoalveolar-lavage fluid, were collected from patients with pneumonia of unknown cause who were identified in Wuhan on December 21, 2019.

Seven bronchoalveolar-lavage fluid specimens were collected from patients in Beijing hospitals with pneumonia of known cause to serve as control samples.

Extracted nucleic acid samples were tested for viruses and bacteria by polymerase chain reaction (PCR), samples were analyzed for 22 pathogens (18 viruses and 4 bacteria) A real-time reverse transcription PCR (RTPCR) assay was used to detect viral RNA by targeting a consensus RdRp region of pan β-CoV.

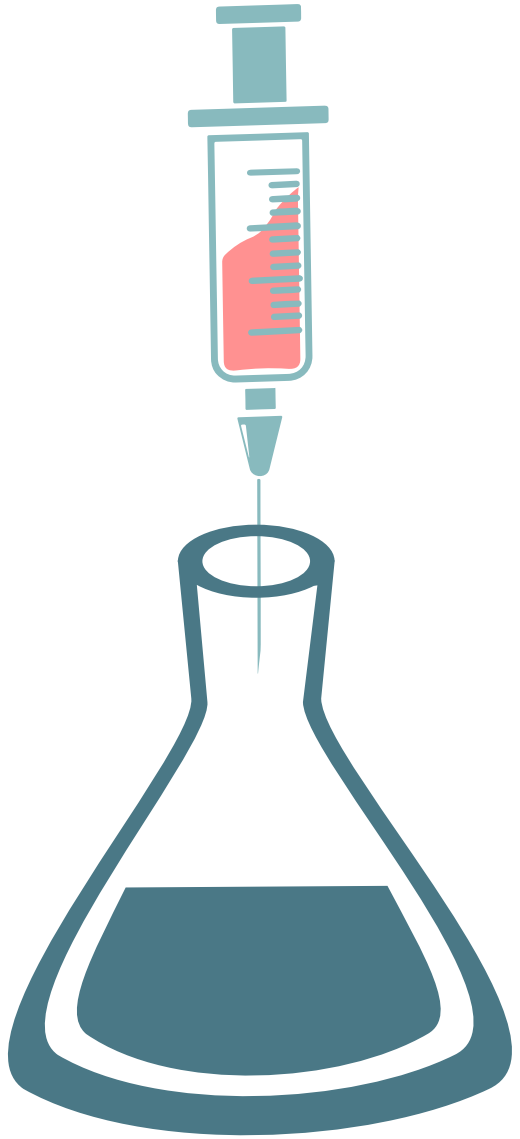


# Isolation of Virus

Bronchoalveolar-lavage fluid samples were collected in sterile cups to which virus transport medium was added. Samples were then centrifuged to remove cellular debris.

The supernatant was inoculated on human airway epithelial cells, which had been obtained from airway specimens from patients undergoing surgery for lung cancer.

Human airway epithelial cells were expanded on plastic substrate, and the cultures were generated in an air–liquid interface to form well-differentiated



# Isolation of Virus

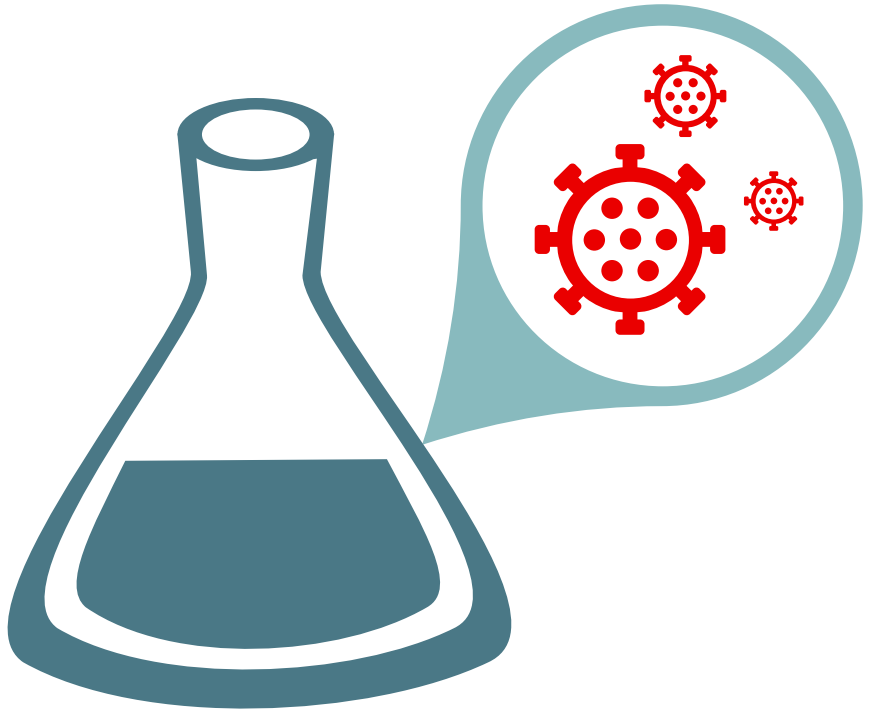
Prior to infection the apical surfaces were washed three times with phosphate-buffered saline. supernatant from bronchoalveolar-lavage fluid samples was inoculated onto the apical surface of the cell cultures. After a 2-hour incubation at 37°C, unbound virus was removed; the cells were maintained in an air–liquid interface incubated with carbon dioxide. phosphate-buffered saline was applied to the apical surfaces every 48 hours and after 10 minutes of incubation the samples were harvested.

# Isolation of Virus

Pseudostratified mucociliary epithelium cells were maintained in this environment; apical samples were passaged in a 1:3 diluted vial stock to new cells.

The cells were monitored daily:

- ✓ Light microscopy for cytopathic effects
- ✓ RT-PCR for the presence of viral nucleic acid in the supernatant.



# Transmission Electron Microscopy

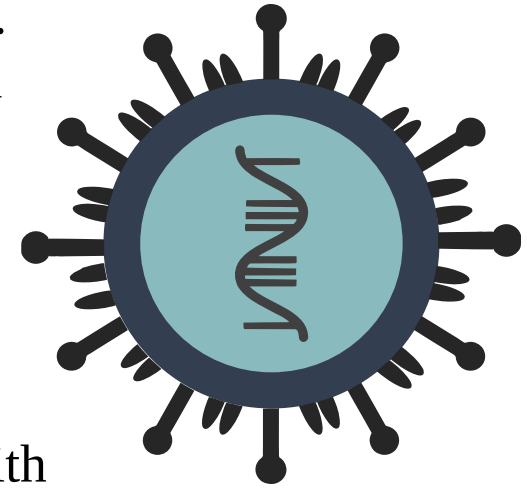
Supernatant was collected and inactivated and ultracentrifuged to sediment virus particles. The enriched supernatant was negatively stained on film-coated grids for examination. Then it fixed with paraformaldehyde, glutaraldehyde and were then fixed with osmium tetroxide dehydrated with grade ethanol embedded with PON812 resin.

A section were cut from resin block and stained with uranyl acetate and lead citrate. The negative stained grids and ultrathin sections were observed under transmission electron microscopy.

# Viral Genome Sequencing

RNA was used as a template to clone and sequence the genome. We used a combination of Illumina sequencing and nanopore sequencing to characterize the virus genome.

Sequence reads were assembled into contig maps with the use of CLC Genomics software. Specific primers were subsequently designed for PCR, and 5′- or 3′-RACE was used to fill genome gaps from conventional Sanger sequencing.



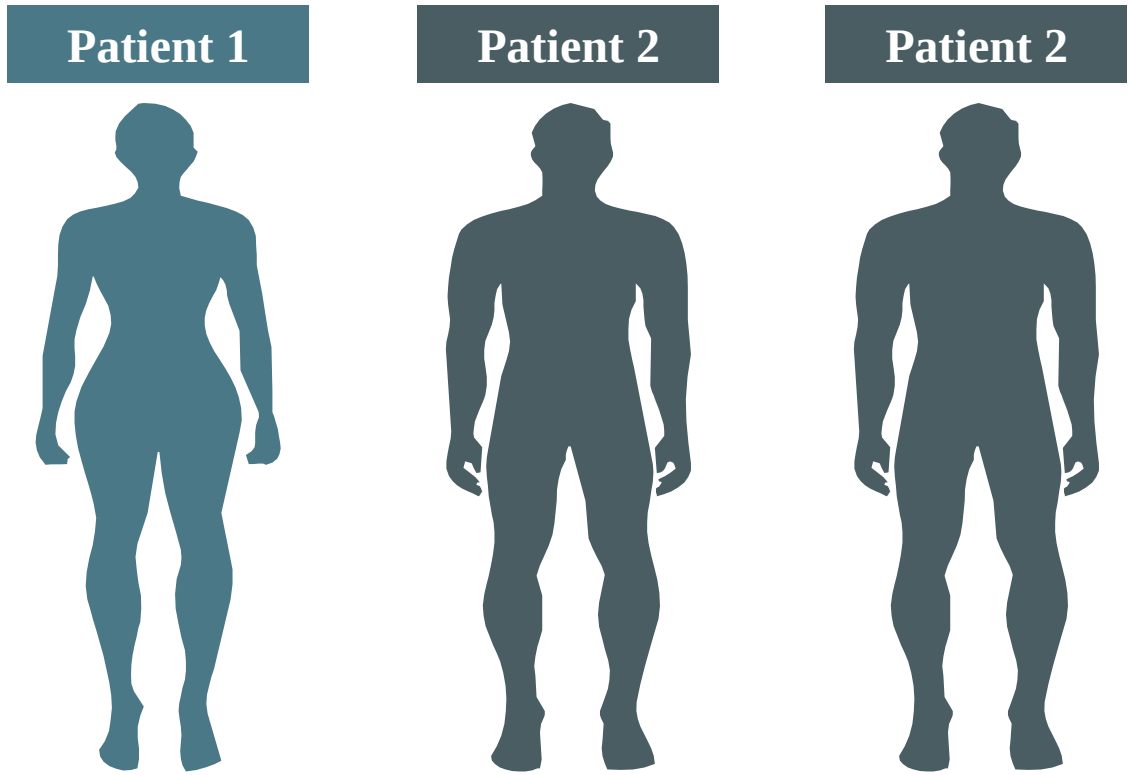
These PCR products were purified from gels and sequenced, in accordance with the manufacturers' instructions.

Multiple-sequence alignment of the 2019nCoV and reference sequences was performed with the use of Muscle. Phylogenetic analysis of the complete genomes was performed, and a general time-reversible model used as the nucleotide substitution model.

# Result

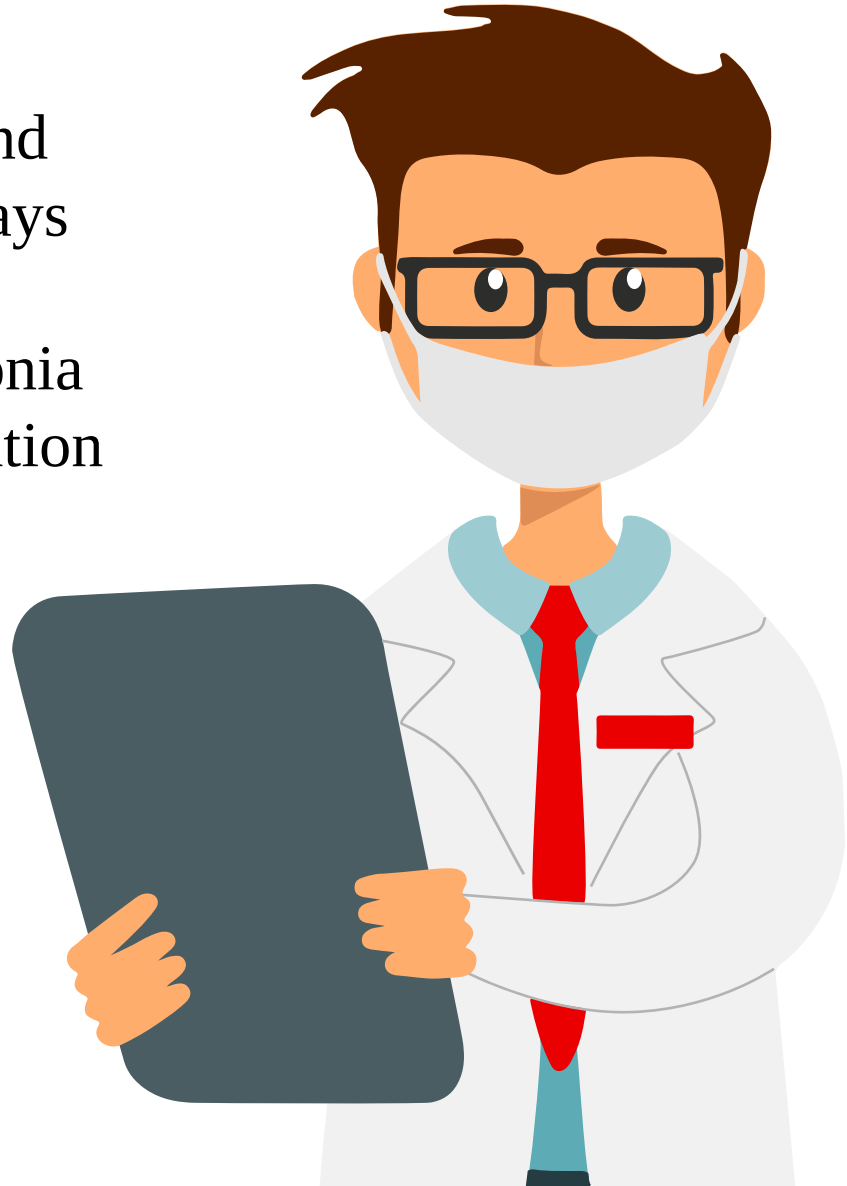
Three adult patients presented with severe pneumonia and were admitted to a hospital in Wuhan on December 27, 2019.

- Patient 1 was a 49-year-old woman
- Patient 2 was a 61-year-old man
- Patient 3 was a 32-year-old man



# Result

Patient 1 reported having no underlying chronic medical conditions but reported fever (temperature, 37°C to 38°C) and cough with chest discomfort on December 23, 2019. Four days after the onset of illness, her cough and chest discomfort worsened, but the fever was reduced; a diagnosis of pneumonia was based on computed tomographic (CT) scan. Her occupation was retailer in the seafood wholesale market.

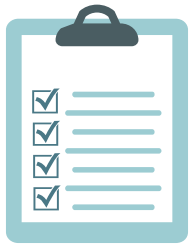


# Result

Patient 2 initially reported fever and cough on December 20, 2019; respiratory distress developed 7 days after the onset of illness and worsened over the next 2 days

Three bronchoalveolar-lavage samples were collected from Wuhan Jinyintan Hospital on December 30, 2019. No specific pathogens were detected in clinical specimens from these patients by the Respi Finder Smart- 22kit.

Positive results were also obtained with use of a real-time RT-PCR assay for RNA targeting to a consensus RdRp region of pan β-CoV



# Discussion

- ✓ Evidence for the presence of this virus includes identification in bronchoalveolar-lavage fluid in three patients by whole-genome sequencing, direct PCR, and culture.
- ✓ The illness likely to have been caused by this CoV was named “novel coro- navirus-infected pneumonia" (NCIP).
- ✓
- ✓ Complete genomes were submitted to GISAID.
- ✓ Phylogenetic analysis revealed that 2019-nCoV falls into the genus betacoronavirus, which includes coronaviruses (SARS-COV, bat SARS-like CoV, and others) discovered in humans, bats, and other wild animals.

# Discussion

Ad- novel beta coronavirus that is likely to have been dictional evidence to confirm the etiologic sighted cause of severe pneumonia in three patients nuisance of 2019-nCoV in the Wuhan outbreak in Wuhan, China.

Although establishing human airway epithet the lung tissue of patients by immunohistolial cell cultures is labor intensive, they appear chemical analysis, detection of IgM and IgG to be a valuable research tool for analysis of huantiviral antibodies in the serum samples from man respiratory pathogens.

# Reference


- Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R. and Niu, P., 2020. A novel coronavirus from patients with pneumonia in China, 2019. New England Journal of Medicine

# THANK YOU

For listening