3D image of SARS-CoV-2 infected lungs

The blue portion of the lung is the more normal part; the yellow part is lung being destroyed by the virus.

Video tour of an infected lung:

Covid-19 Research Restart

Chemistry Department

Phil Pellett

June 1, 2020

Purpose: To provide background about the virus and the pandemic to provide context for WSU plans to safely restart research and maintain educational progress during the Covid-19 pandemic.

The laboratory research restart was authorized by Governor Whitmer May 15.

Audience: Chemistry Department grad students, plus more

WSU guidance:
https://research.wayne.edu/coronavirus/restartguidance
**Mental health**

Breaks and vacations matter. Discuss with your PI.

Psychological services are available through the university ([https://caps.wayne.edu/](https://caps.wayne.edu/)).

*Don’t wait to ask for help.*

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**Research opportunities related to Covid-19 and SARS-CoV-2**

Tell the story

Legal issues

Socioeconomic disparities – document and address

Clinical – diagnosis and treatment

Public health epidemiology & case tracking

Prevention – vaccine trials, behavioral interventions (what works?)

Antivirals – no BSL3 at WSU, but surrogate systems can be used (Dr. Nguyen)

Drugs to treat Covid-19-related diseases
Covid-19 and SARS-CoV-2 background for lab research restart

- Covid-19 is the disease; SARS-CoV-2 is the virus.
- Basic virology of coronaviruses
- Biological properties of coronaviruses and how they have contributed to the pandemic
- Layers of protection
- Roles of diagnostic tools in clinical and pandemic management
- Paths to treatment and prevention.
- $R_0$, and why we must keep it suppressed.

Covid-19 in the US

May 31

Ascertainment bias -- undercounting

https://www.worldometers.info/coronavirus/
Covid-19 internationally

Coronavirus Cases:
6,263,901

Deaths:
373,899

Recovered:
2,846,713

Ascertainment bias -- undercounting

https://www.worldometers.info/coronavirus/

Covid-19 timecourse

Scale change
The disease (Covid-19), the virus that causes it (SARS-CoV-2), and how their properties affect safety protocols

The disease

• Upper respiratory tract and lung infection leads to transmission via coughing, singing, speech, and breathing.

• Intestinal infection leads to presence of the virus in sewage (>10^7 virus genomes per liter in Paris sewage).

• Sites of infection: cells protected by mucous membranes (oropharynx, lungs, eyes)

• Range of disease: asymptomatic or very mild to fatal (asphyxiation, multi-organ clot-related disorders, cytokine storm)

• ~20,000 new cases per day in the US.

Defining properties of viruses (1)

- Metabolically inert on their own.
- Infectious, obligate intracellular parasites.
  - Dependent on cellular systems
  - Co-evolved with cells
- Virus genomes consist of either DNA or RNA.
- Virus genomes direct their own replication and the synthesis of other viral components, using cellular systems in an appropriate host cell.
- Virus particles (virions) are formed by assembly from newly synthesized components within the host cell.
- Virions are vehicles for transmission of virus genomes to the next host cell or organism
- Virion disassembly initiates the beginning of the next infectious cycle.
Defining properties of viruses (2)

- Biomechanical devices
- Associated with all forms of life
- Genetic information in a deliverable package
- Minimal virus: genome with origin of replication, plus a coat
  - DNA or RNA genome
  - Proteinaceous coat
    - capsid
    - nucleocapsid
    - capsid that is a virion substructure
  - envelope
- Virion
- Translation parasites
  - major distinction from bacteria

The virus: SARS-CoV-2

A coronavirus

In electron micrographs, coronavirus virions have a halo or crown ("corona") made up of the virion surface spike protein, which interacts with cell surface molecules (virus receptors) at the step of virus entry.

The spike protein is embedded in a lipid bilayer that is derived from host cell membranes (an enveloped virus).

- 30 kb ssRNA genome that can express ~27 proteins
- genome can serve as a mRNA (plus-strand RNA virus)
  - >10 proteins are cleaved from a polyprotein of ~7000 amino acids by viral proteases that are part of the polyprotein.
    - The proteases are targets of antiviral development (Dr. Kovari and collaborators).
Virions are SMALL!

One teaspoon (5 ml) of laboratory-grown virus stock can hold more coronavirus virions than there are people in the United States.
### Virus Classification

**RNA viruses**

- **Symmetry of capsid**
  - Isometric
  - T=3
  - T=4
  - T=5
  - T=6
  - T=7
  - T=8
  - T=13
  - T=15
  - T=17
  - T=19
  - T=21
  - T=27
  - T=30

**Major criteria:**
- DNA or RNA genome
- Nature of capsid
- Enveloped or non-enveloped (naked)

**DNA viruses**

- **Symmetry of capsid**
  - Isometric
  - T=3
  - T=4
  - T=5
  - T=6
  - T=7
  - T=8
  - T=13
  - T=15
  - T=17
  - T=19
  - T=21
  - T=27
  - T=30

**Major criteria:**
- DNA or RNA genome
- Nature of capsid
- Enveloped or non-enveloped (naked)

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**Electron microscopy of SARS-CoV-1 virions**

These images provided the first evidence that the 2003-2003 SARS epidemic was caused by a coronavirus. Note the crown-like projections that form a corona at the periphery of virions.

**Figure 2.** Ultrastructural Characteristics of SARS-Associated Coronavirus Grown in Vero E6 Cells.

Panel A shows a thin-section electron-microscopical view of viral nucleocapsids aligned along the membrane of the rough endoplasmic reticulum (arrow) as particles bud into the cisternae. Enveloped virions have surface projections (arrowhead) and an electron-lucent center. Directly under the viral envelope lies a characteristic ring formed by the helical nucleocapsid, often seen in cross section. Negative-stain electron microscopy (Panel B) shows a stain-penetrated coronavirus particle with an internal helical nucleocapsid-like structure and club-shaped surface projections surrounding the periphery of the particle, a finding typical of coronaviruses (methylamine tartrate stain). The bars represent 100 nm.

Ksiazek et al., NEJM 2003
Coronavirus virion

Virion MW $\sim 40 \times 10^6$

Moderate environmental stability

Coronavirus virion

Envelope (lipid bilayer plus glycoproteins)

Genome coiled with nucleoprotein

Spike protein

Genome is $\sim 30,000$

Nucleotides of plus-strand RNA

**Glycosylation of SARS-CoV-2 Spike protein**

https://www.acrobiosystems.com/A1117-The-Art-of-glycosylation-of-SARS-CoV-2-S-Protein.html

Effects on vaccine performance?

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**SARS-CoV-1 genome**

~30 kb RNA^+^  

The plus-strand genome serves as an mRNA for translation of pp1a and pp1ab, when are then proteolytically cleaved into numerous smaller proteins (shown above).

After synthesis of the negative strand, shorter subgenomic plus-strand transcripts are generated that enable translation of additional proteins (right side, from the Spike protein (S), downward.

**Four enzymes:** 2 proteases, a helicase, and the RdRP

Philip E. Pellett, Ph.D.
Origins of SARS-CoV-2

Across most of the sequence, the most closely related virus is Bat CoV (RaTG13).

- ~96% identity between RaTG13 and SARS-CoV-2
- ~1200 nucleotides differ between the viruses
- Not a single evolutionary jump.

Zhang et al.  
J. Proteome Research  
https://dx.doi.org/10.1021/acs.jproteome.0c00129
Droplet vs. airborne (aerosol) transmission

SARS-CoV-19

EBOLA

3 feet

Large droplets fall from air

SARS-CoV-19

MEASLES

6 feet

Small droplets remain in air (aerosol)

Protective equipment

We wear seat belts, but seldom need them.
• >25% of the price of a car is for safety features.

Lab coats, gloves, eye protection

Masks
• Areas of uncertainty and disagreement
  • Use of masks is strongly correlated with reduced rates of infection.
    • https://www.preprints.org/manuscript/202004.0203/v2
  • Bilateral protection (not just masks)
  • What kind of mask?
• How to don and doff masks
  https://www.google.com/search?q=how+to+doff+an+N95+mask&rlz=1C1GCEU_en&ei=how+to+doff+an+N95+mask&qgs=chrome_6957016_7479qj8&sourceid=chrome&ie=UTF-8#kpvalbx=_igTCXsC8KZq6Iqaq94uQAw35

Labs have high air turnover.
Remember the Paris sewers (go before you go).
Layers of protection for yourself and others while at work

Without training and commitment, the restart will crash and burn.

Residual risk

\[
\text{Residual risk} = 0.20 \times 0.75 \times 0.95 \times 0.95 \times 0.20 \times 0.50 = \text{~1\% residual risk}
\]

You control most of the variables.

Going viral: viral transmission network

\[ R_0 \] – number of individuals infected by each case

http://wwwnc.cdc.gov/eid/article/12/11/06-0255-f4
Reducing $R_0$: Why social distancing matters (1)

$R_0$ - The basic reproduction number for infectious agents

- If $R_0 \leq 1$, the infection will disappear.
- If $R_0 \geq 1$, the infection will continue to propagate through the population.
- $R_0$ for measles varies from 12-18, ~4 for SARS-CoV-1, 2 for HIV and HCV.

How is the $R_0$ of a viral disease calculated?

Infectious period (little human control over this; antivirals can help)
- Can vary with age of patient, etc.
- Viruses can differ widely, e.g., HSV-2 (lifetime) vs. norovirus (one day)

Rate of contact with susceptible individuals (humans can control this)
- Social distancing reduces $R_0$.
- Vaccination and large numbers of recovered individuals reduces $R_0$.

Mode of transmission (mild human control over this)
- Close personal interaction (HSV-2) vs. dispersal via coughing

Why social distancing matters (2)

Complexity of the basic reproduction number ($R_0$)

- $R_0$ is an estimate of contagiousness that is a function of human behavior and biological characteristics of pathogens.
- $R_0$ is not a measure of the severity of an infectious disease or the rapidity of a pathogen’s spread through a population.
- $R_0$ values are nearly always estimated from mathematical models, and the estimated values are dependent on numerous decisions made in the modeling process.
- The contagiousness of different historic, emerging, and reemerging infectious agents cannot be fairly compared without recalculating $R_0$ with the same modeling assumptions.
- Some of the $R_0$ values commonly reported in the literature for past epidemics might not be valid for outbreaks of the same infectious disease today.

from Delamate et al., Complexity of the basic reproduction number ($R_0$), Emerg. Infect. Dis. Jan. 2019

- $R_0$ changes as variables such as the susceptible fraction of a population changes. We can influence this.
Covid-19 and SARS-CoV-2: disinfectants

The virus does not replicate outside of living mammalian cells.

**Enveloped virion**
- Disinfectants disrupt the envelope and capsid.

**Disinfectants**
- [https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html](https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html)
- EPA-approved hydrogen peroxide-based surface disinfectants
- EPA-approved quaternary ammonium compounds
  - Remain on surface
  - Persist on surfaces, some adverse health effects
- 70% ethanol or 70% isopropanol are OK if EPA-approved disinfectants are not available.
  - Bleach (sodium hypochlorite) – effective disinfectant if fresh, pits stainless steel

Covid-19 and SARS-CoV-2: Treatment and prevention

**Self-defense**
- Intrinsic, innate, and adaptive immunity
- Self-defense plus support

**Support**
- Breathing
- Prevention of coagulopathy

Antivirals are in development (Dr. Kovari).

Vaccines are in development.

**Social distancing and case tracking matter**
- ~1% of the population has experienced the virus.
- *Need to avoid overloading hospitals.*
- Some populations are at very high risk.
Are we safe with respect to Covid-19?

Risk in our laboratories is directly related to our behaviors outside the lab.

Imperfect and imprecise predictions for risk; random happens

Opinions vs. facts. It is important to make judgements based on facts.

Safety questions and concerns can be turned into useful homework and group discussions within labs.

We must answer safety questions in a data-driven matter.

OEHS is our friend.

The way forward

SARS-CoV-2 is a new, highly transmittable and highly pathogenic virus that is capable of quickly being moved internationally. Perhaps 1% of the US population has been infected thus far. That means 99% of us are still susceptible.

Development, manufacture, and distribution of a vaccine is unlikely in less than two years.

Things needed before relaxing the need for social distancing:

• PCR testing for the virus needs to return results within four hours of specimen collection. First-responders and healthcare workers need to be tested regularly, and on demand.
• Serosurveys need to be done to understand the frequency of asymptomatic infections, as well as the prevalence of exposed, and hopefully protected individuals. This is underway.
• PPE, medical equipment, and toilet paper pipelines need to be re-filled.
• Clinical staff in hard-hit areas need a break.
• Hopefully, an efficacious antiviral regimen is identified quickly.