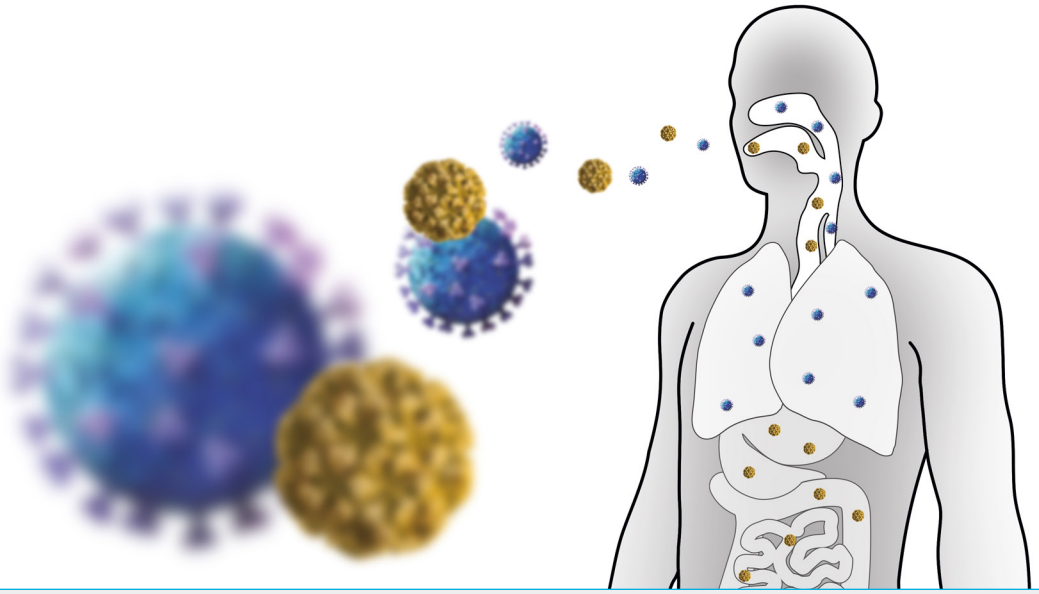
BIO-RAD Explorer



Virus Detection and Transmission Kit
(Coronavirus)

Catalog #17008261EDU

Student Guide

For technical support, call your local Bio-Rad office, or in the U.S. call 1-800-4BIORAD (1-800-424-6723) option 2.

BIO-RAD

Contents

Outbreak of a Respiratory Disease

A new respiratory disease has been reported recently in the region surrounding AnyTown, USA. Called Pneumonia-Like Syndrome[1] (PLS), this disease is spreading rapidly and causes symptoms that include shortness of breath, cough, severe body aches, and high fever. PLS has led to numerous hospitalizations, and patients are not responding to standard treatments for pneumonia or influenza (the flu).

Scientists have applied the latest **genome sequencing** methods to find that PLS is caused by a coronavirus (CoV) they call PLS-CoV. Coronaviruses are a large family of viruses that can cause illnesses that range in severity, from a common cold to COVID-19. PLS-CoV is considered a "novel" coronavirus because its genomic sequence is quite different from all other strains previously found in nature. Because PLS-CoV is novel, little is known about how it spreads, how it causes disease, or how it should be treated. There is no vaccine and no immunity within the population, so everyone is susceptible to infection.

As PLS-CoV spreads, doctors, researchers, and clinicians are working as quickly as possible to understand the **pathophysiology** of PLS-CoV and to find the most effective treatments. Molecular biologists have used the PLS-CoV **genome sequence** to develop a diagnostic test that can detect PLS-CoV infections. Virologists, molecular biologists, epidemiologists, and public health officials are also working together to understand the nature of this virus and how it spreads.

Figuring out the **mode of transmission** will be key to deciding the best way to keep the spread under control.

In this series of activities, you will gain insights into the variety and range of expertise needed to diagnose, study, and mitigate virus outbreaks.

- You will begin as an emergency room physician, where you will diagnose two patients who have different symptoms that are consistent with PLS
- When one of the patients tests positive for PLS-CoV infection, you will then move into the role of a medical lab scientist and perform a PLS-CoV diagnostic test on samples collected from other suspected cases
- Next, to determine the mode of transmission of this virus, you work as an epidemiologist and consider all the data you have about an outbreak of PLS at a local restaurant
- Finally, you take on the role of a public health official who must decide whether to take immediate action to control the spread of this disease and the best means of doing so

[1] Please note this is a fictitious disease spread by a fictitious virus. For information about how viruses and the diseases they cause are named, refer to Appendix A.

Activity 1

Learning about Virus Biology, Pathophysiology, and Detection

In this activity, you will learn about viruses, how they infect humans and cause disease, and the challenges doctors may face when diagnosing patients based on symptoms alone. You will then explore how diagnostic tests work and how they can help doctors make a definitive diagnosis.

Part 1: Patient Symptom Review

It is late in the evening of Sunday, June 8, and you are an emergency room (ER) physician at AnyTown Hospital. Two patients arrive at the ER within an hour of each other. Both are experiencing symptoms that are characteristic of Pneumonia-Like Syndrome (PLS) (Figure 1).

- Patient A, a 23-year-old male, has a nagging, dry cough, body aches, and fever of 104°F.
- Patient B, a 46-year-old female, has a nagging cough and severe congestion.

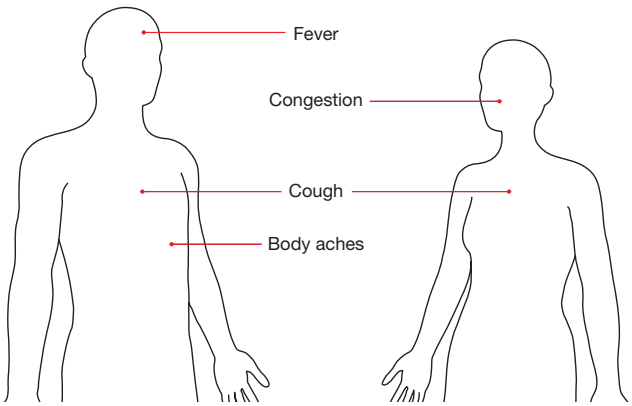


Figure 1. Patients A (left) and B (right).

As the ER physician, you examine both patients. Pneumonia is an infection that inflames the air sacs in one or both lungs. It can be caused by a bacterial, viral, or fungal infection. You'd like to determine whether the patients are suffering from a bacterial or a viral infection, or whether the source of their symptoms is something else, such as allergies. You find their lungs sound clear of any infection, and there are no signs of bacterial infection in the patients' ears or throats. Based on the patients' symptoms and the recent concern over the regional uptick in PLS cases, you suspect both patients may be suffering from a viral infection, possibly PLS-CoV.

Review the symptom chart (Table 1) and answer the Focus Questions that follow.

Table 1. Symptom chart for several respiratory ailments. For this activity, consider only these ailments. In reality, there may be many other causes for the respiratory symptoms of Patients A and B.

Symptoms	PLS (Pneumonia-Like Syndrome)	Common Cold	Influenza (Flu)	Allergies
Fever or chills	Common	Sometimes	Common	Never
Cough	Common	Common	Common	Sometimes
Congestion or runny nose	Common	Common	Common	Common
Sneezing	Rare	Sometimes	Sometimes	Common
Shortness of breath or difficulty breathing	Common	Rare	Common	Sometimes (asthma-related)
Fatigue	Common	Sometimes	Common	Sometimes
Muscle or body aches	Common	Sometimes	Common	Never
Headache	Common	Sometimes	Sometimes	Rare
Sore throat	Common	Common	Common	Rare
Nausea or vomiting	Sometimes	Never	Sometimes	Sometimes (possible with food allergies)
Diarrhea	Sometimes	Never	Sometimes	Sometimes (possible with food allergies)
Diagnostic tests available	Yes	No	Yes	Yes
Treatments available	Unknown; patient isolation recommended	Over-the-counter analgesics, decongestants, and cough suppressants	Prescription antivirals and over-the-counter analgesics, decongestants, and cough suppressants	Over-the-counter antihistamines, decongestants

Focus Questions

Based on each patient's symptoms, which respiratory ailment(s) in Table 1 might they have? Which would you rule out as unlikely, and why?

Based on the patients' symptoms, which diagnostic test(s) would you order for each patient?

PLS is a new and potentially lethal viral disease that is spreading rapidly. What are potential outcomes from not testing/determining the actual cause:

- ***For the patient?***

- ***For the community?***

Part 2: Learning about Viruses, Pathophysiology, and Detection

The Biology of Viruses

Viruses are everywhere. They infect all life forms, from bacteria to plants to animals, in every ecosystem on Earth. In fact, viruses are the most numerous biological entities. Millions of types of viruses are believed to exist, but only 9,000 virus species have been described in detail.

We are exposed to viruses every day, though certain environments or actions can increase the likelihood of exposure or infection. The study of viruses is called **virology**, and it is a subspeciality of microbiology.

Viruses cannot move on their own, have no metabolism (they do not "eat" anything), and they cannot reproduce by themselves. Instead, viruses infect living cells and hijack the reproductive and metabolic machinery of those cells to make more copies of themselves. In doing this, viruses can cause disease.

Virus particles are made up of the same types of molecules that characterize all living cells: proteins, lipids, carbohydrates, and nucleic acids. At their most basic, they consist of nucleic acid surrounded by a protein coat known as a **capsid** (the capsid + genome combination is called a **nucleocapsid**). Some viruses may also have a membrane that surrounds the nucleocapsid, called an **envelope**, and they may contain enzymes needed to infect host cells. A complete viral particle with all the components needed for host cell infection is called a **virion** (Figure 2).

Viruses contain all the genetic material needed for host cells to make new virions, and this full complement of genetic material is known as its **genome**. Whereas the genomes of some viruses, like chickenpox and smallpox, are made of DNA like those of humans (a virus that has a DNA genome is classified as a **DNA virus**), others are made of RNA (**RNA virus**). Because of their propensity to **mutate**, RNA viruses are usually the ones we hear about as emerging and causing concern over (new) diseases. These mutations help viruses adapt to new host species.

A virus's genomic sequence is its molecular fingerprint. Scientists use a virus's genomic sequence to determine its identity and its genetic relatedness to other known viruses.

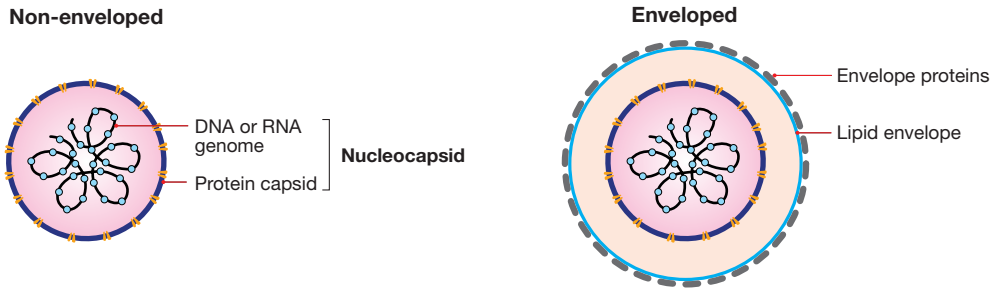


Figure 2. Structure of a virion. Non-enveloped viruses (left) do not have the lipid membrane that enveloped viruses (right) have.

Career Spotlight

Virologist

Virologists are research scientists who study viruses. They work in academic, industrial, or government institutions and contribute to core knowledge about viral biology — their genetic makeup, structure, hosts, infection cycle, etc. This knowledge guides the work done by doctors, pathologists, and others to prevent and rapidly respond to viral disease outbreaks.

Entry-level technician positions are available to those with a college degree; postgraduate degrees and research experience are needed for those wanting to run their own research programs.

Mechanisms of Infection and Disease

How Viruses Infect the Human Body

When viruses infect cells, they hijack those cells to turn them into virus-making factories. This process has many variations, but in general, the key stages of infection include the following:

- **Virus enters the body** — viruses enter the human body through a portal of entry (Figure 3A), usually the eyes, respiratory tract (including mouth, nose, lungs), urogenital tract, gastrointestinal (alimentary) tract, or skin (for example, through a needle or insect or animal bite)
- **Virus attaches to a host cell** — cells of the respiratory, gastrointestinal, skin, and genital tissues are the most common sites of infection. There, proteins on the cell surface (receptors) interact with proteins on the outside of the virus; the specific interaction between a viral protein and a host cell receptor protein determines which species a virus can infect (Figure 3B, step 1)
- **Virus penetrates the host cell** — once a virus binds a receptor, it deposits its genome into the host cell. The way this happens depends on the virus: some inject their DNA directly into the cell, others are taken up by endocytosis, or (as in the case of enveloped viruses) through fusion of the lipid envelope with the host cell membrane (Figure 3B, step 2)
- **Virus replicates** — the virus takes control of the host cell and directs it to express the viral genes to produce viral proteins and assemble more virions (Figure 3B, steps 3–5)
- **Progeny viruses exit the host cells** — the hundreds to thousands of progeny virions then leave the host cell (a process that often leads to cellular damage or death) to infect other cells (Figure 3B, step 6). Progeny virions may infect neighboring cells to cause a localized infection, or they can spread through bodily fluids into different organs
- **Virus sheds into the environment** — virions eventually spread to a portal of exit (for example, the respiratory, gastrointestinal, and urogenital tracts and blood) and are released into the environment; the virus then goes on to be transmitted to another person to set up a new infection cycle

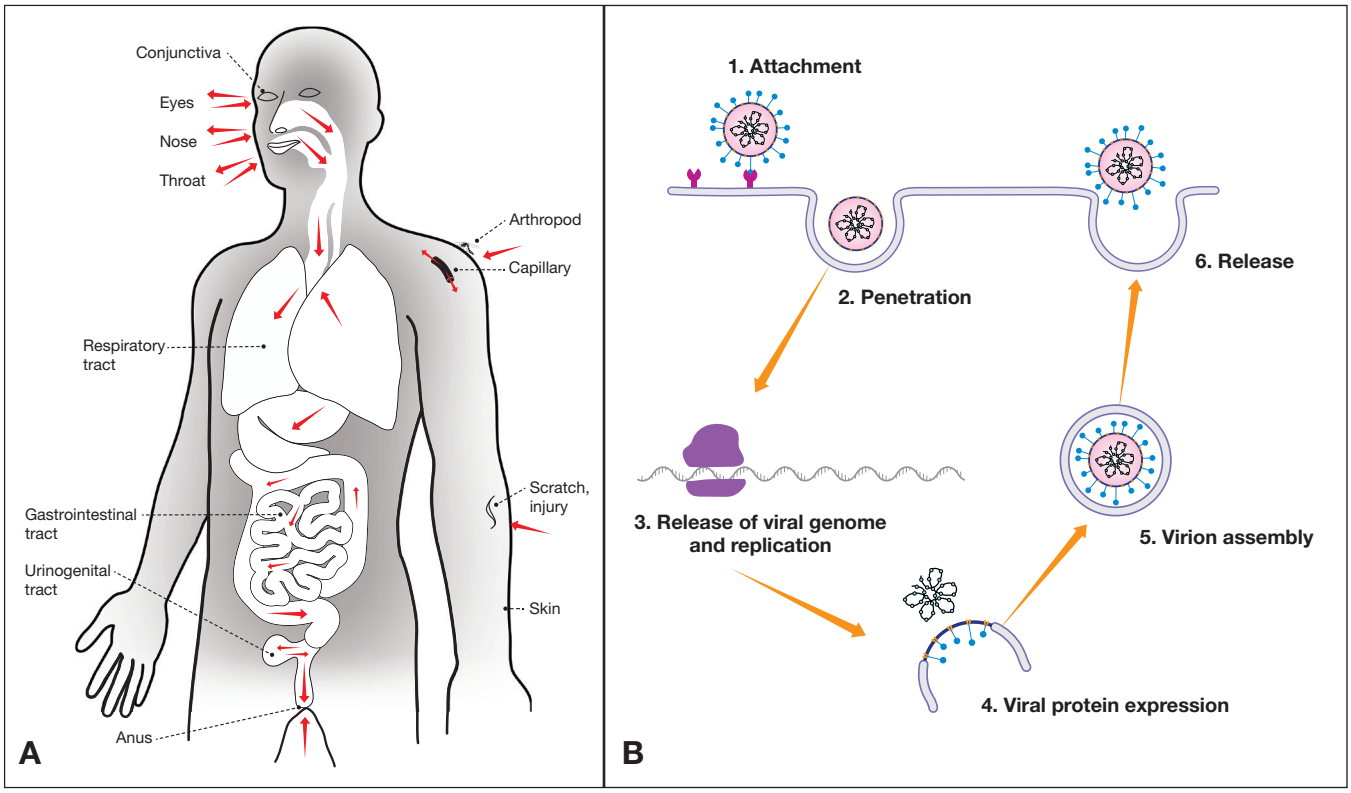


Figure 3. Portals of entry into a human host (A) and the stages of viral infection of a host cell (B).

How Viruses Cause Disease (Pathophysiology)

For most people most of the time, the immune system can handle a viral infection. In some cases, though, viral infections can cause disease in several ways:

- ***Damage caused by the virus*** — viruses can destroy infected host cells by interrupting cellular functions, by releasing enzymes and other proteins that degrade cellular components, or by lysing the cell to cause the release of progeny virions. This can cause damage to tissues and organs and make us sick. Some viruses, like the human papilloma virus (HPV), can also integrate their DNA into the host to cause damage later in the form of cancer or other diseases
- ***Damage caused by the immune system*** — as viruses infect a host, they stimulate the immune system, which then destroys the virus and infected cells; if this response goes too far, it may also lead to severe pathological consequences to the host, like excessive inflammation or targeted cell death
- ***Damage caused by a secondary infection*** — cells, tissues, and organs that are damaged by a virus or immune response may become more vulnerable to secondary infections. For example, a common cold can be the result of a viral infection, but a patient may develop a bacterial infection like bronchitis, sinusitis, or pneumonia as a secondary effect

Two Common Viruses

This activity centers around a coronavirus outbreak. However, two distinct groups of viruses are presented here to demonstrate how different viruses can behave differently in the human body.

Coronavirus (CoV)

Coronaviruses (CoVs) have risen to fame and notoriety as a group of viruses that can cause serious respiratory syndromes in humans. In fact, there are many different types of CoVs, and they are common in many different mammals and in birds. When they infect humans, some strains of CoV can cause colds or other mild respiratory (nose, throat, lung) illnesses. Others, however, can cause serious respiratory diseases, including severe acute respiratory syndrome (SARS, caused by SARS-CoV), Middle East respiratory syndrome (MERS, caused by MERS-CoV), and coronavirus disease 2019 (COVID-19, caused by severe acute respiratory syndrome coronavirus 2, or SARS-CoV-2).

Refer to Appendix A for information about how viruses and the diseases they cause are named.

CoVs are RNA viruses that have a lipid envelope. CoV virions are spherical, have an average diameter of 125 nm, and are named for their appearance: “corona” means “crown,” and the virus’s outer layers are covered with spike proteins that surround them like a crown (Figure 4). These spike proteins help virions bind to and infect cells by acting as molecular “keys to the host cell” — they bind to receptor proteins on the surface of the host cell, which allows the virus to enter the host cell. Beneath these spikes is the lipid envelope, which can be disrupted by detergents and alcohols.

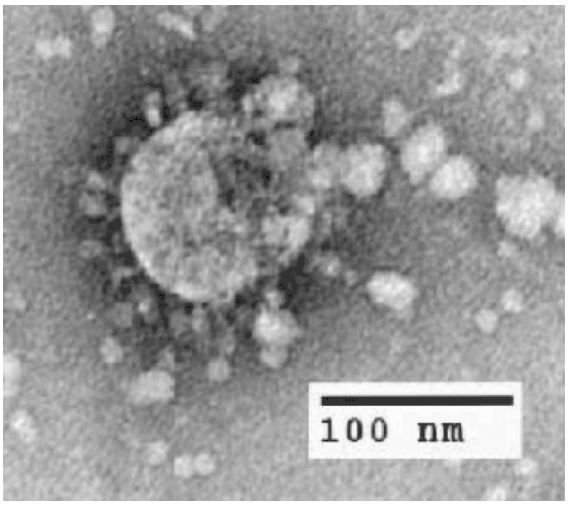


Figure 4. SARS-CoV particle. Negative stain electron microscopy of a SARS-CoV virion. Note the characteristic club-shaped projections surrounding the periphery of the virion. Source: C.D. Humphrey, CDC (https://www.cdc.gov/sars/lab/images.html).

Two of the most famous CoVs, SARS-CoV and SARS-CoV-2, infect humans primarily through the respiratory tract and by binding with the ACE2 receptor protein. ACE2 receptors are found all over the human body but are particularly dense in intestinal epithelial cells, the lungs, blood vessels, endothelial and smooth cells of the blood vessels and heart, and the tubular epithelial cells of the kidneys (Figure 5).

Norovirus (NoV)

Noroviruses (NoVs) are another group of RNA viruses. Unlike the CoVs, however, they are not enveloped by lipids, and this makes them more stable on surfaces. The NoV protein coat helps it withstand detergents and even acids. Strong disinfectants like bleach are needed to disrupt and inactivate NoVs.

NoV virions are 30–40 nm in diameter, and humans and other mammals serve as natural hosts.

Upon infection, NoVs bind to histo-blood group antigens (HBGAs), which line the gastrointestinal tract (Figure 5). Also referred to as the winter vomiting bug or just stomach bug, NoVs are the most common cause of gastroenteritis (infectious diarrhea), which is characterized by non-bloody diarrhea, vomiting, stomach pain, and sometimes fever or headaches.

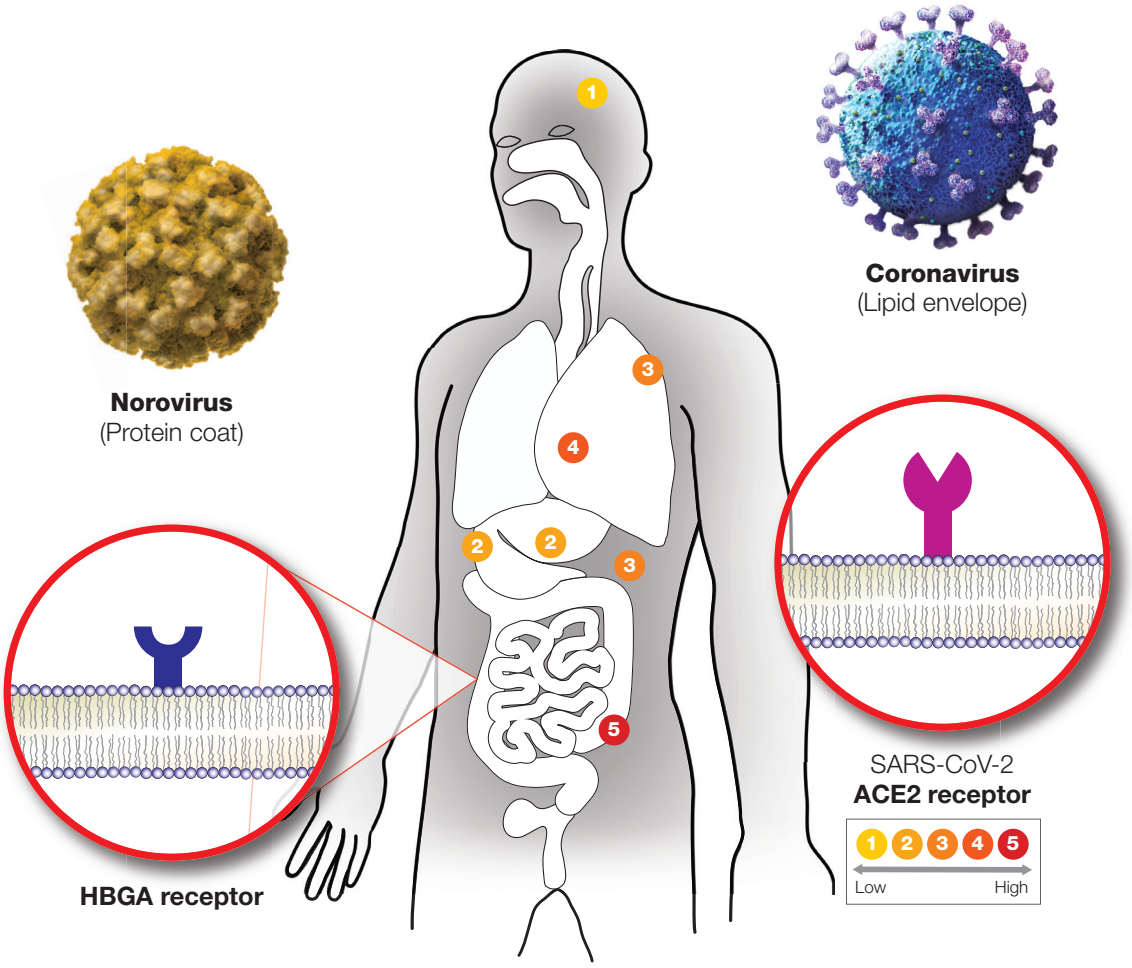


Figure 5. Viral pathogenesis. Different viruses target different tissues based upon receptor preference. Noroviruses bind to HBGA receptors, which occur primarily in the gastrointestinal tract. The SARS-CoV-2 virus binds to ACE2 receptors, which are found in various tissues and organs throughout the body. In this image, the color of ACE2 receptor sites reflects the relative abundance of those receptors (for example, there are higher levels of ACE2 receptors in the lungs and gastrointestinal tract than in the liver or brain).

Focus Questions

Would you call a virus a "living cell"? Why or why not?

Using the information in Figure 5, explain why SARS-CoV-2 can cause symptoms other than respiratory distress (for example, gastrointestinal symptoms).

Which virus would you expect to remain intact longer on a doorknob: a CoV or a NoV?

Career Spotlight

Pathologist

Pathologists are medical doctors who specialize in the causes, nature, and effects of disease. Pathologists hold medical degrees (MD) with a specialty in pathology and work mostly in hospitals and medical, academic, or industrial laboratories. Though pathologists are a part of a patient's healthcare team and are integral to patient diagnosis and treatment, they may never actually meet the patient.

Career Spotlight

Histotechnician and histotechnologist

Histotechnicians and histotechnologists are medical laboratory technicians who prepare tissue samples for examination by a pathologist. Histotechnicians are trained to prepare samples for analysis under a microscope. Histotechnologists may also perform testing on tissues and alternative preparation methods, and they may supervise other staff. Histotechnicians and histotechnologists work in hospitals or clinical pathology labs, research laboratories, doctors' offices, for pharmaceutical companies, or for government agencies.

Histotechnicians need vocational education, an associate degree (from a community college), or a high school diploma and two years of related work experience. To become a histotechnologist, you will need to have a college degree and certification.

Detecting Viruses in Patient Samples

Diagnosing patients based on their symptoms alone can be difficult. Different conditions often cause similar symptoms, and those symptoms may overlap with other medical conditions. For example, a stuffy nose and sneezing may be caused by the common cold or by allergies. When diagnosing a viral infection, a doctor considers all the symptoms a patient is experiencing as well as their recent activity and exposure history.

To make a conclusive diagnosis, doctors often also rely on a diagnostic test. Diagnostic tests exist for a variety of infections (for example, bacterial, fungal, protist). This activity focuses on the diagnosis of viral infections. There are two general types of diagnostic tests for viral infections:

- **Antigen or antibody tests** — use antibodies to detect the presence of virus particles, viral proteins, or a patient's own antibodies against the infection:
  - An **antigen test** uses antibodies to detect virus particles or viral proteins in patient samples; it can detect an active infection (an example is the at-home rapid antigen test used to detect SARS-CoV-2/COVID-19)
  - An **antibody test** uses antibodies to detect other antibodies in patient samples; it can detect whether a person has had an infection in the past
- **PCR-based tests** — use PCR amplification to detect the presence of a viral genome in the patient's bodily fluids or tissues:
  - **PCR tests** for DNA viruses
  - **Reverse-transcription PCR (RT-PCR) tests** for RNA viruses; they include an added step to convert the RNA into DNA before PCR (Figure 6)

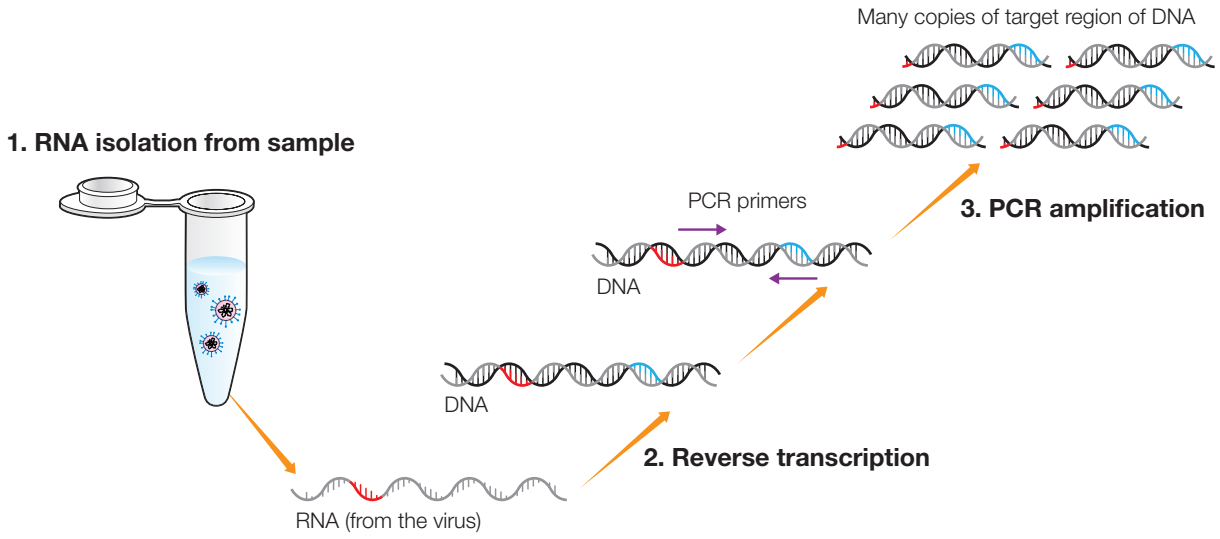


Figure 6. Reverse-transcription PCR (RT-PCR). RT-PCR uses an enzyme called reverse transcriptase to reverse the transcription process and convert RNA back into DNA. The DNA then serves as the template in a PCR. In this diagram, the purple arrows on the DNA represent primers, short sequences of DNA that direct PCR amplification of a specific target region on the DNA.

Focus Questions

Antigen tests are also used to test for pregnancy. In these tests, the antigen being detected is human chorionic gonadotropin (HCG), a protein hormone whose presence in urine over a certain level indicates a pregnancy. Can a PCR-based test be used to detect HCG?

Which of the four types of tests listed above could you use to test for an active CoV or NoV infection?

Medical Laboratory Professional

Medical laboratory science professionals perform laboratory analyses that help physicians in patient diagnosis, treatment, monitoring, and disease prevention. Their training and expertise can include clinical chemistry, hematology, immunology, microbiology, and molecular biology. They consult with other members of a healthcare team.

Medical laboratory technicians often have an associate degree or certification and perform the collection, processing, and analysis of biological specimens. Medical laboratory scientists have a 4-year college degree and certification and therefore a deeper theoretical knowledge base. They perform laboratory procedures and help interpret the results, consult with medical teams, conduct research, and develop new test methods.

Part 3: Patient Diagnosis

As the ER physician, you suspect that Patient A and Patient B may be infected with the novel coronavirus, PLS-CoV, but their symptoms also do not rule out influenza. You order diagnostic tests for influenza and for PLS-CoV.

About the PLS-CoV Test

The AnyTown Hospital has access to the new RT-PCR test that can detect the presence of PLS-CoV in human samples.

The lab technician collects small samples of cells from the nasopharynx of Patients A and B using **nasopharyngeal swabs** (Figure 7). This a common sample collection method for tests that detect bacteria or viruses that cause respiratory infections.

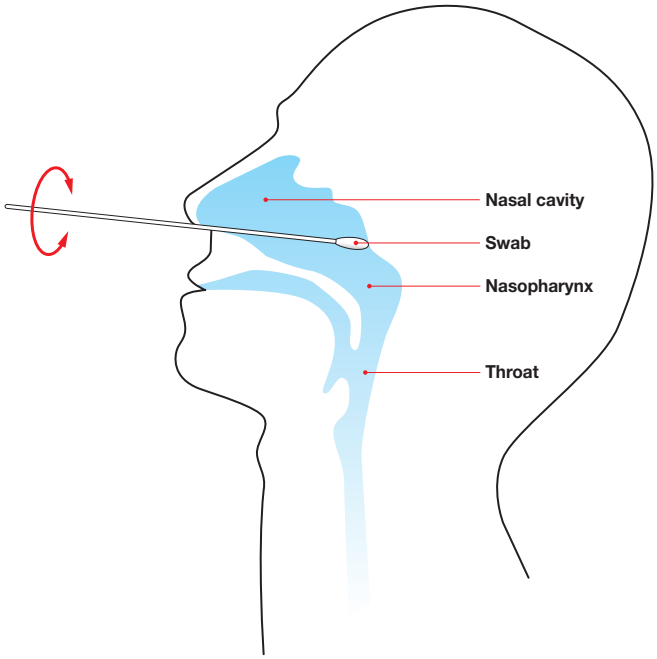


Figure 7. Collection of a sample using a nasopharyngeal swab.

After the sample is collected, a medical laboratory technician performs the PLS-CoV RT-PCR test. This test uses PCR amplification of two genes (Table 2):

- **A gene specific to the virus PLS-CoV** — the test detects the *N* gene, which encodes the nucleocapsid protein of PLS-CoV. The test is designed to detect only a specific section of the *N* gene of PLS-CoV. It will not detect *N* genes from any other CoVs that may be present
- **A gene found in all human samples** — in this case, *GAPDH* is used; this serves as a control to confirm successful extraction and amplification of nucleic acids from human samples

Table 2. Target regions of the PLS-CoV diagnostic test.

Target region	Band size	Purpose
PLS-CoV *N* gene	~530 bp	Detects presence of PLS-CoV
Human *GAPDH* gene	~250 bp	Control: confirms that nucleic acid was successfully collected and extracted from a human source

In this test, two sets of primers are mixed in the same tube with the sample: one set will bind to and amplify the *GAPDH gene*, and one set will bind to and amplify the *N* gene. In this way, technicians can look for both genes in the same sample.

Following PCR, the medical laboratory technician analyzes the samples by **agarose gel electrophoresis**. The term **electrophoresis** refers to the movement of charged molecules in response to an electric field, which can be used to separate them. In this case, the technique is used to separate DNA pieces by size (Figure 8) and allows them to be visualized.

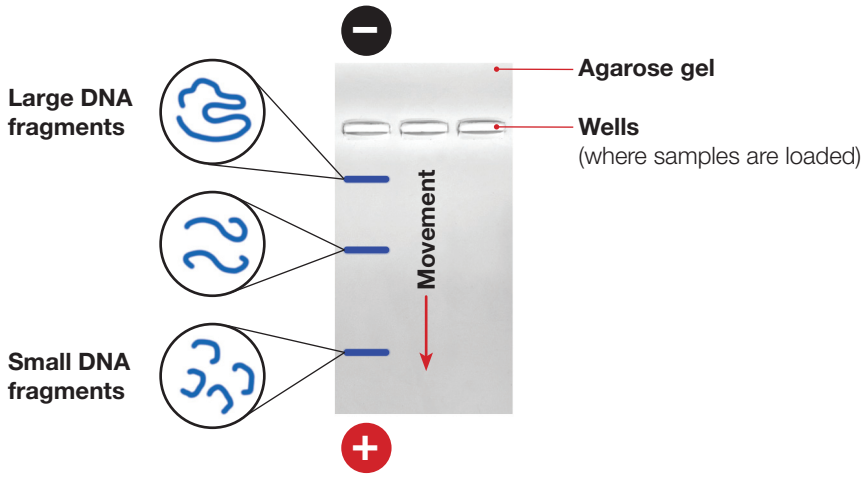


Figure 8. Agarose gel electrophoresis. By convention, the rectangular spaces across the top of a gel represent the wells into which samples are loaded. The negatively charged DNA fragments migrate through the gel toward the positive pole (anode) when a voltage is applied. The straight path the DNA fragments follow through the gel is called a lane. Smaller fragments migrate more quickly through the gel matrix and so they move farther into the gel than larger fragments.

For the PLS-CoV test, the technician loads the RT-PCR products (DNA fragments) into an agarose gel slab. The agarose gel is in an electrophoresis cell and covered in a conductive buffer solution. When the technician starts the electrophoresis, current flows between the electrodes at each end of the chamber (Figure 8). The negatively charged electrode (cathode) is closest to the sample wells, and when electrophoresis starts, the DNA fragments move toward the positively charged electrode (anode) because DNA carries a net negative charge.

As the DNA fragments move, the agarose gel acts as a sieve, or a matrix of holes. Smaller DNA fragments move more easily than larger ones, so the smaller DNA fragments travel farther than larger ones (Figure 8). The relative size of fragments contained in each band can be found by measuring how far each band has traveled from the wells.

An analogy for gel electrophoresis is a situation where all the desks and chairs in a classroom are randomly pushed together. An individual student can wind their way through the maze quickly and with little difficulty, but four students holding hands would require more time and have difficulty working their way through the maze. Smaller chains of students will move (migrate) through the maze more quickly, and so move farther through the maze in each period than larger chains.

The nasopharyngeal swab sample from all patients contains any infecting virus as well as some human cells that have sloughed off during sample collection. In this PLS-CoV test, if the RNA sample was successfully collected, extracted, and amplified:

- All human patient samples should have a band for the *GAPDH* gene
- Only patients infected with PLS-CoV will also have the *N* gene band, which is specific to PLS-CoV

Focus Questions

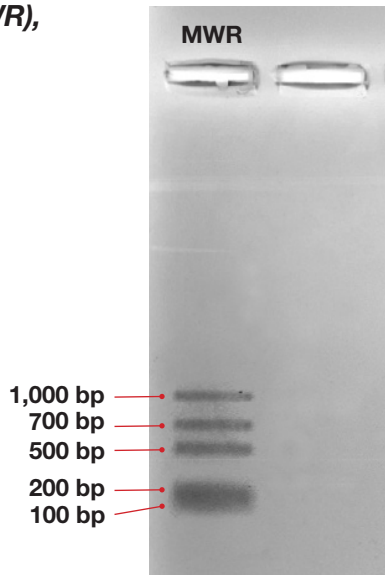
In the PLS-CoV test, which target region — the N gene or GAPDH gene — would produce a band that would move farther than the other by agarose gel electrophoresis? Why?

How many bands would you expect to see in a sample collected from a person who is infected with PLS-CoV?

In the gel at right, the first lane has a molecular weight ruler (MWR), which is a set of DNA fragments of known size. These fragments are used to estimate the size of DNA fragments in the samples.

In the second lane, draw and label the bands you would expect to see in the PLS-CoV tests for:

- ***The amplified N gene target***
- ***The amplified GAPDH gene target***



Patient Data

Now that you understand how the PLS-CoV test works, you move into the role of medical lab scientist at AnyTown Hospital. You will review the test results for Patients A and B (Figure 9) and communicate the test results back to the ER physician.

Your analysis includes the following samples:

- **MWR** — molecular weight ruler with DNA fragments 1,000, 700, 500, 200, and 100 bp
- **(–) Control** — this control has only the *GAPDH* gene; it simulates a sample from a person who is not infected with PLS-CoV
- **(+) Control** — this control has both the *N* and the *GAPDH* genes; it simulates a sample from a person who is infected with PLS-CoV
- **NTC (no-template control)** — this control is a sample that has only the PCR primers and no DNA template
- **Patient A**
- **Patient B**

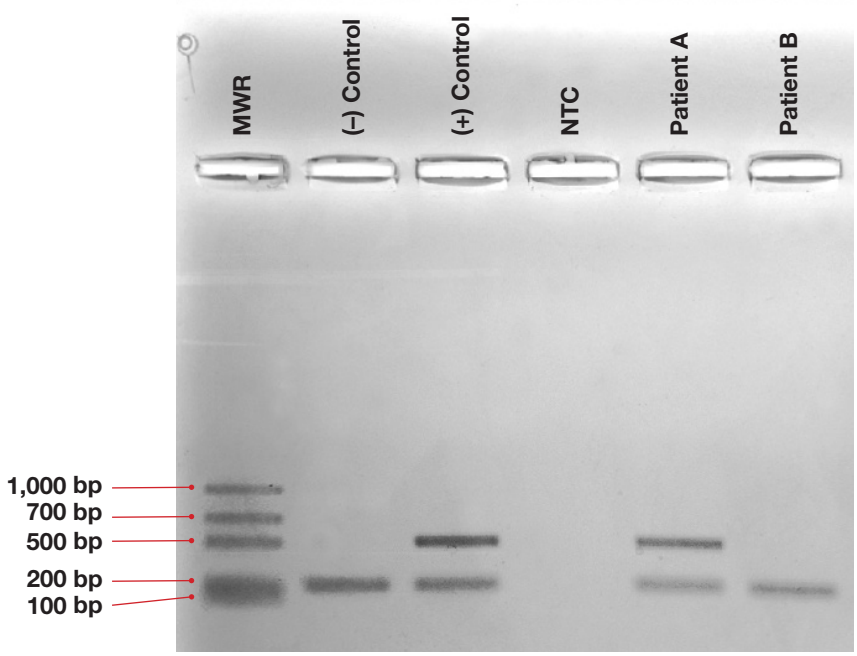


Figure 9. Agarose gel electrophoresis of the controls and patient samples from the PLS-CoV diagnostic test. This gel shows the bands obtained from an RT-PCR test, including primers that amplify the PLS-CoV *N* gene and the human *GAPDH* gene. MWR = molecular weight ruler; (–) control = control sample containing the human *GAPDH* gene; (+) = positive control sample containing both the *N* and *GAPDH* genes; NTC = no-template control (containing no template DNA); A = sample from Patient A; B = sample from patient B.

Focus Questions

Is either patient infected with the virus? How do you know?

Is either patient not infected with the virus? How do you know?

What would you conclude from a sample if it did not have the GAPDH band?

What would you conclude from an analysis in which there was a band in the NTC lane?

Activity 2
Detecting Infections

As the medical lab scientist at AnyTown Hospital, you reported the confirmed case of PLS-CoV infection to local public health officials. The next day, the officials took a detailed patient history for Patient A and, to contain the spread of PLS-CoV, they initiated contact tracing to notify all known contacts of Patient A of their exposure and ask them to be tested.

Before he showed up with symptoms at the AnyTown Hospital in the late evening of June 8, Patient A had been at the AnyTown Restaurant for lunch. At the time, he was experiencing only a mild cough, but by the evening, his symptoms had worsened, and he was in the ER. After notification from the hospital of the positive case, the public health officials contacted the AnyTown Restaurant to track down the other customers and staff who were in the restaurant for lunch on June 8. The officials interviewed and collected nasopharyngeal swabs from each person for diagnostic testing.

In this activity, you act as a medical lab technician and use DNA gel electrophoresis to analyze the PCR samples of all the people who were in the restaurant at the same time as Patient A. You will then combine your results with results from your classmates to figure out which, if any, other restaurant staff and patrons are infected with the novel CoV, PLS-CoV.

Part 1: Pre-Laboratory Questions

You have four of the restaurant staff/patron samples for your analysis. You will combine your results with those from your class to get a complete picture of the infection status of all restaurant staff and patrons.

Your analysis will include the samples listed in Table 3. Before you begin, fill in the Purpose column in Table 3 with the details of each sample in your analysis.

Table 3. Samples included in the PLS-CoV diagnostic test.

Sample	Description	Purpose
MWR	Molecular weight ruler	
(–)	Control sample containing the *GAPDH* gene	
(+)	Control sample containing both the *N* and *GAPDH* genes	
NTC	No-template control (water; no DNA)	
Restaurant samples	Four staff/patron samples designated by a number	

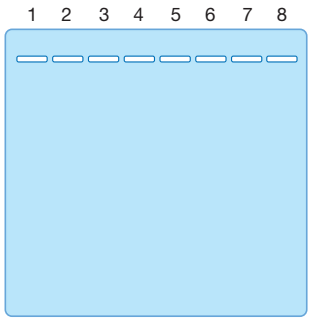
Part 2: Patient Sample Analysis by Agarose Gel Electrophoresis

Student workstation	Quantity
Molecular weight ruler (MWR), 20 µl	1
GAPDH control (–), 10 µl	1
N gene and *GAPDH* positive control (+), 10 µl	1
No-template control (NTC), 10 µl	1
Samples from restaurant staff and patrons, 10 µl	4
TAE electrophoresis buffer	300 ml
100x Fast Blast DNA Stain (if using)	50 ml
1% TAE agarose gel with 8 wells	1
Horizontal gel electrophoresis chamber	1
Power supply (may be shared)	1
Microcentrifuge tube rack	1
Micropipet and tips	1
Gel staining tray (optional)	1
Waste container	1

Protocol

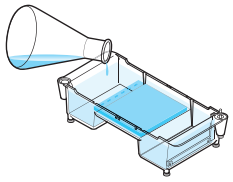
Running DNA on an Agarose Electrophoresis Gel

1. Sketch your agarose gel setup to the right. Label each of the eight wells with the sample that will be loaded.



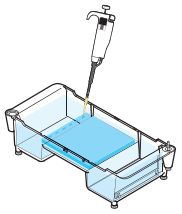
2. Place a 1% TAE agarose gel into the electrophoresis chamber. Be sure that the gel is oriented so that the wells are closest to the black (–) electrode, or cathode.

3. Fill the electrophoresis chamber with enough TAE buffer to cover the gel by about 2 mm.

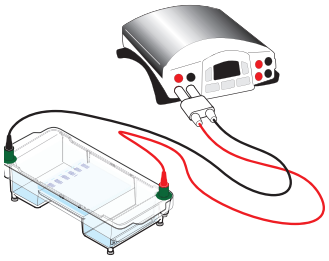


4. Using a fresh pipet tip for each sample, load 10 µl of each DNA sample and 20 µl of MWR into each gel according to the table below.

Lane	Sample
1	MWR, 20 µl
2	(–), 10 µl
3	(+), 10 µl
4	NTC, 10 µl
5	Restaurant patron or staff sample, 10 µl
6	Restaurant patron or staff sample, 10 µl
7	Restaurant patron or staff sample, 10 µl
8	Restaurant patron or staff sample, 10 µl



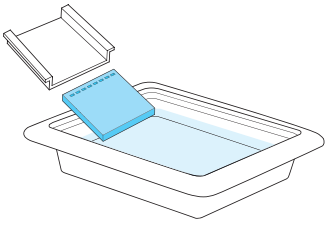
5. Place the lid on the electrophoresis chamber and connect the electrical leads to the power supply, red to red and black to black.



6. Turn on the power and run the gel. Ask your instructor for the run conditions.

7. When the electrophoresis run is completed, turn off the power and remove the lid from the chamber.

8. Carefully remove the gel from the electrophoresis chamber and transfer it as directed by your instructor. Be careful — the gel is very slippery.



9. Stain and/or visualize your gel as directed by your instructor.

Part 3: Analysis

1. Complete the table and sketch your electrophoresis results. Label the bands of the molecular weight ruler using the sizes shown in Figure 9.

Lane	Sample
1	
2	
3	
4	
5	
6	
7	
8	

2. State the results of the control samples and describe what you can conclude from them.

3. Record your restaurant staff/patron results below.

Person ID	Infection Status (Positive/Negative/Undetermined)

Part 4: Collecting Class Data

Gather the results from other groups in your class. In the table below, record the infection status for all who were in the restaurant.

Table 4. Class results.

Person	Infection Status (Positive/Negative)	Person	Infection Status (Positive/Negative)
A1		C6	
A2		C7	
A3		E1	
A4		E2	
B1		E3	
B2		E4	
B3		E5	
B4		F1	
B5		F2	
B6		F3	
B7		F4	
B8		F5	
C1		F6	
C2		W1	
C3		W2	
C4		K1	
C5			

Activity 3
Building a Transmission Model

In this activity, you assume the role of epidemiologist. You will integrate all the diagnostic test data with other information about the restaurant patrons and staff to figure out how PLS-CoV spread throughout the restaurant.

Career Spotlight

Epidemiologist
Epidemiologists study how disease outbreaks start, how diseases are transmitted, and how to effectively mitigate spread. This involves collecting and analyzing data, communicating findings, and working with other programs and agencies to decide courses of action. Their findings influence regulatory, private, and public policy.

Epidemiologists typically earn a postgraduate, master's degree in public health, focusing on epidemiology. Epidemiologists find employment at state or local health departments, hospitals, colleges, universities, and federal government agencies, such as the Centers for Disease Control and Prevention (CDC).

Part 1: Understanding the Chain of Infection

To stop a **contagious disease** from spreading, you first need to find out how it is spreading. Infectious disease control and prevention rely on understanding the chain of infection.

Chain of Infection

When epidemiologists talk about infectious diseases like PLS, they describe a chain of infection that includes six points (Figure 10):

- **Infectious agent** — the pathogen (for example, virus, bacterium, fungus); this activity focuses on the virus PLS-CoV as the infectious agent
- **Reservoir** — the environment in which the infectious agent lives (for example, people, animals, insects, etc.)
- **Portal of exit** — the point at which the infectious agent leaves the reservoir; in humans, this refers to where on the body the infectious agent leaves
- **Mode of transmission** — the way the infectious agent moves on to a new host
- **Portal of entry** — where on the body the infectious agent enters a new host
- **Susceptible host** — person who can be infected

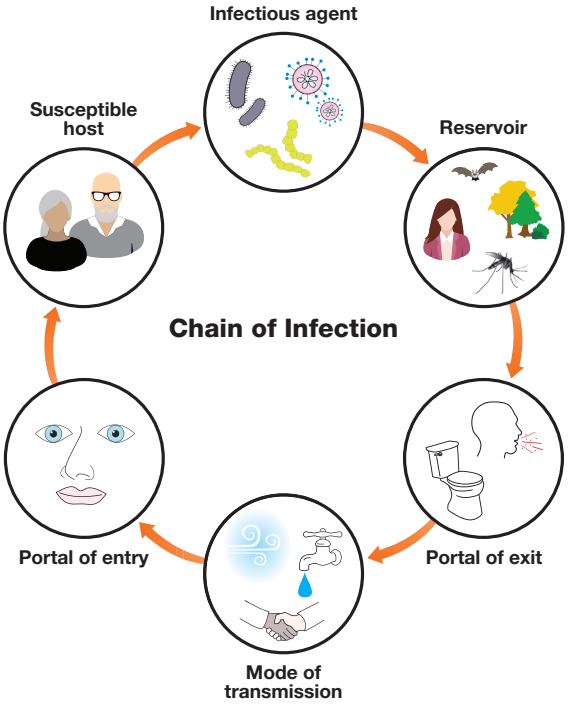


Figure 10. Chain of infection. In epidemiology, a chain of infection has six main points.

The chain of infection can be broken wherever it is vulnerable, most often at:

- **Controlling or eliminating the pathogen at transmission**, for example by stopping the pathogen (in this case, PLS-CoV) from spreading
- **Protecting portals of entry** — preventing the pathogen from entering a host, for example by wearing a mask
- **Increasing host's defenses** — making the host more resilient to infection, for example by vaccinating the host

Reservoirs

A reservoir is where a pathogen lives, replicates, and spreads. Reservoirs include:

- **Humans** — the reservoirs for many infectious diseases, humans can carry infections and pass them on to others while being **symptomatic** (showing physical symptoms of disease) or **asymptomatic** (showing no symptoms but still carrying and transmitting viruses)
- **Animals and insects** — infectious diseases transmitted from animals/insects to humans are referred to as **zoonotic** diseases
- **Environment** — soils, water, and contaminated foods may also harbor pathogens that can spread to humans

Identifying reservoirs can be a critical component of fighting pathogen-caused disease. For example, it was possible to **eradicate** smallpox because humans were the only reservoir for the smallpox virus. Naturally occurring smallpox was eradicated after the last human case was identified and isolated. It is much more difficult to control or eradicate viruses that have multiple reservoirs or hosts.

Portals of Entry and Exit

To cause disease, a pathogen must access tissues and cells through a portal of entry. To spread to others (for disease transmission to occur), it must leave the host through a portal of exit (refer to How Viruses Infect the Human Body, Activity 1).

The portals of exit and entry may be the same in a host. For example, a virus may exit from the nose or mouth when a person exhales, talks, laughs, or sneezes. It may then enter the nose or mouth of a new host when that person inhales. Or the portals of entry and exit might be different. For example, virions may exit one person through their feces or vomit, be carried along on unwashed hands to food, water, or utensils and enter a new host through its mouth.

Modes and Routes of Transmission

Viruses can move between individuals in many ways that are categorized as either direct or indirect (Table 5).

- **Direct transmission** occurs directly between individuals through direct, person-to-person contact or droplets
- **Indirect transmission** typically occurs via an intermediate such as a surface, a vector like mosquitoes, or **airborne** particles; the reservoir and new host do not need to be near each other for transmission to occur

Some viruses may use more than one mode of transmission.

Table 5. Modes of transmission.

Direct	Indirect
Contact • Skin-to-skin contact like holding hands • Direct exchange of bodily fluids through kissing or sexual intercourse **Droplets** from sneezing, coughing, or even talking; these droplets are large (>5 µm), have a limited range of transmission, and quickly fall to the ground	**Fomites** (contaminated inanimate objects) like door handles, cell phones, shared drinking glasses, surfaces, or other objects **Contaminated food or water** **Vectors** such as mosquitoes or ticks **Aerosols/airborne** small liquid droplets (<5 µm) from sneezing, coughing, or even talking; these droplets can remain suspended in the air for extended periods of time (up to several hours)

Whether a virus has a lipid envelope or not is a key factor in determining its route of transmission.

Enveloped viruses require an intact lipid envelope to be able to infect, and they must remain in a wet environment to keep the lipid envelope intact. So enveloped viruses typically spread through respiratory droplets in a **respiratory-aerosol route** or in blood via insect bites, injection, or even organ donations.

When infected people exhale, talk, laugh, sing, cough, or sneeze, they spray mucus and other bodily fluids that can contain viruses or other pathogens (Figure 11). These fluid drops vary in size from larger liquid **droplets** (>5 µm) to smaller liquid **aerosols** (<5 µm)[2]. Whereas the larger droplets have a shorter range for transmission because they quickly fall to the ground, smaller liquid aerosols can remain suspended in the air (airborne) for longer periods of time and so accumulate in the air and travel for greater distances, especially when there is poor ventilation.

[2] There is no absolute cutoff in size to help distinguish a droplet from an aerosol; this is a topic of debate among experts (Nicas et al. 2005, Siegel et al. 2007, Tellier, 2009, Gralton et al. 2011).

Small aerosols are more susceptible to be inhaled deep into the lungs to cause infection in the lower respiratory tract, while large droplets tend to be trapped and cause infections in the upper airways.

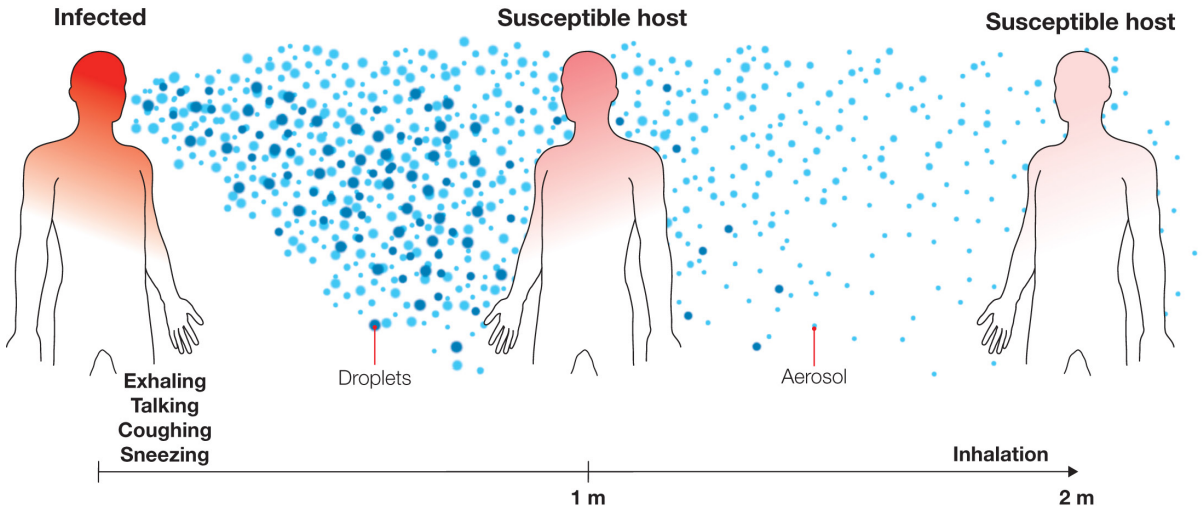


Figure 11. Droplet and airborne transmission. When an infected person exhales, talks, coughs, or sneezes, virus-laden drops are transported through the air, where a potential host may inhale them to initiate a new infection. The smaller the droplet, the farther it may drift. Larger droplets (>5 µm) tend to fall quickly out of the air and so require direct transmission through direct contact, or indirect transmission as they fall on and contaminate surfaces (fomites). In contrast to droplets, aerosols are much smaller (<5 µm), can linger in air for hours and travel beyond 1 to 2 m from the infected individual who exhales them, causing new infections at both short and long ranges.

Virus-containing droplets and aerosols can also be generated from nonrespiratory activities, such as toilet flushing or vomiting.

Non-enveloped viruses can withstand extremes of pH, detergents, and dryness. They can even withstand the harsh pH and detergent conditions within the gastrointestinal tract and so can be transmitted through a **fecal-oral route**, in which virions enter through the mouth and exit through feces to contaminate another surface and infect others.

Host Susceptibility

Once a virus enters a host, the susceptibility of that host depends on multiple factors that include species (some viruses can infect only a particular species while others can affect a broader range), age (the youngest and/or oldest in a population will be most susceptible), and overall health and immunity status (whether the host has been vaccinated against infection or has been infected previously).

Understanding whether host susceptibility is tied to age or sex, for example, can help public officers understand how best to control spread. Host susceptibility can also be a target for prevention strategies like vaccination or therapies.

For pathogens that spread from person to person, once a susceptible person becomes infected, they become a new source for subsequent spread.

Focus Questions

Droplet and aerosol transmission both involve viruses (or other pathogens) that are suspended in bodily fluids. Why, then, is droplet transmission considered direct and aerosol transmission considered indirect?

Enveloped viruses have exterior lipid membranes that can dry out in the environment. Which of the modes of transmission listed in Table 5 is least likely for enveloped viruses, and why?

Part 2: Creating a Transmission Model

Public health officials need to know how PLS-CoV is transmitted to figure out how to keep further transmission in check. Your task is to analyze the complete set of data from the patient and restaurant information supplied by your teacher, and to propose an explanation for how it spread.

Instructions

1. ***On the restaurant diagram:***
    a. ***Mark which restaurant staff and patrons were positive (use the class results).***
    b. ***Add other information from the patient cards that you think might be relevant. What did they eat? Did they move about the restaurant?***
2. ***Propose a mode and route of transmission for PLS-CoV at the restaurant. Closely examine the restaurant layout, seating chart, patient movement, and other available data. Remember PLS-CoV is a novel coronavirus and so may have a different mode of transmission from other CoVs.***
3. ***Explain how the evidence supports your proposal.***
4. ***Build a model to propose how PLS-CoV may have spread through the AnyTown Restaurant. Include as many of the six points of the chain of infection in your model as you can.***
    a. ***Can you confidently pinpoint the source(s) of the infection? If yes, who/what was it and how do you know? If no, what other information would you need?***
5. ***Considering your model, do any of the positive or negative test results surprise you? How might you explain those results?***
6. ***How might you test your model of how the virus spread?***

Activity 4
Mitigating Risk

It is now June 15 in AnyTown, and PLS is spreading rapidly through the town and surrounding region. Since the initial diagnosis of Patient A, dozens more patients have been hospitalized with PLS, and a handful are in the AnyTown Hospital intensive care unit (ICU). Doctors are still struggling to find effective treatments.

In this activity, you take on the role of Director for the AnyTown Department of Public Health. After reviewing the goals and strategies for mitigation, it will be your responsibility to decide whether to take measures to slow further spread of the PLS-CoV virus in AnyTown and what those measures might be.

Public Health Director
Public health directors manage local, state, and even national public healthcare programs and organizations with the goal of improving the health and well-being of a population. Public health directors ensure that laws are adhered to as they design and monitor emergency response plans and hold hearings on public health issues. Public health directors have a master's degree or Ph.D. in Public Health and a state license to practice medicine.

Public Health Response

The public health response to a disease outbreak depends on several factors, including whether the virus is novel (for example, PLS-CoV) or common (for example, flu), whether effective treatments are available, contagiousness and severity of the disease caused, and the populations that are affected (severe disease in young children, for example, can be more alarming because treatments may be less available and their bodies more vulnerable to long-term damage).

Novel, highly contagious viruses like PLS-CoV are of particular concern because impacted populations will have no pre-existing immunity. This means the effects of infection may be severe and any available therapies may not be effective. These factors can combine to overwhelm local healthcare facilities, leaving a community unable to respond to other health emergencies or supply preventive care.

When outbreaks of novel viruses occur, epidemiologists and public health officials work together to figure out the best course of action. Epidemiologists tend to focus on figuring out the mode of transmission of a novel virus because once they know how it is transmitted, they will be able to decide what measures to put in place to keep it from spreading. Public health officials work with the epidemiologists, local hospitals, and other agencies to decide which measures to put in place and how to monitor the public health threat from the virus.

Often, there is no clear course of action. Public health officials must weigh the need and efficacy of control measures against their unintended impacts on society, such as effects on local economies and on the overall health and well-being of the population.

Part 1: Understanding Response Strategies to Disease Outbreaks

In person-to-person transmission, once a susceptible host is infected, they become a source of transmission to another susceptible host. One infected person can potentially transmit to more than one susceptible host. Infectious diseases that spread easily from person to person are called **contagious diseases**.

The Epidemic Curve

An epidemic curve shows the number of illnesses detected in an outbreak over time (Figure 12). If an infectious disease spreads uncontrollably through a population, the number of infections tends to rise rapidly, followed by a theoretical steep decline as the disease eventually finds fewer people to infect. Though the total amount of time for the infection to pass through may be short, the total number of infected and sick patients at the peak of an outbreak may overwhelm the capacity of local health systems. This concerns public health officials because it could impact the ability of patients to receive care and of noninfected citizens to access their regular healthcare. Eventually, illness may also hit doctors and nurses and impact other essential services upon which a society depends, including police and fire, teachers, grocery store workers, etc.

If, however, protective measures are put in place to control or reduce the rate of spread, the number of infections could theoretically be lowered to a level that does not exceed the health system's capacity. This decreases the risk of overwhelming the health services and gives more time for treatments and vaccines to be developed. In this scenario, the total number of infections might be spread out over a longer period; that is, the infection curve would be "flattened" (Figure 12).

For more information and a graphic illustration of this phenomenon, visit **www.washingtonpost.com/graphics/2020/world/corona-simulator**.

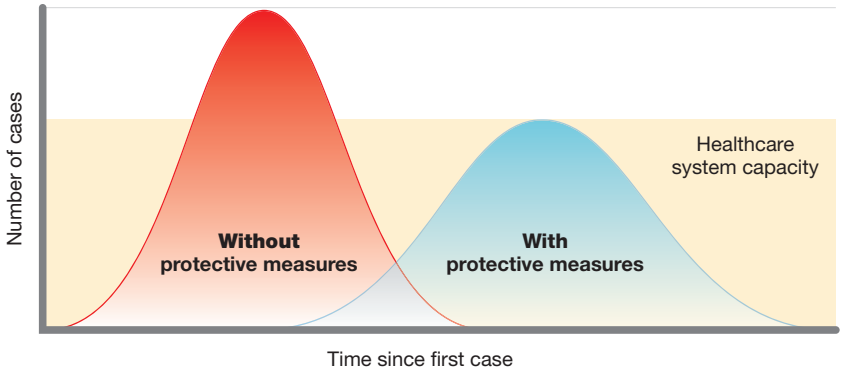


Figure 12. The epidemic curve. The epidemic curve plots the total number of cases of a disease over time. Without protective measures, a highly contagious disease causes the number of infections to increase quickly to a level that may exceed the capacity of a healthcare system. Implementing protective measures can slow the spread of disease and can keep the number of infections within the boundaries of the healthcare system's capacity.

Other Risks of Uncontrolled Spread

As viruses spread, they replicate, and their genes undergo random changes, or genetic mutations. Over time, these genetic copying errors can lead to changes in how the virus behaves (which species or tissues it infects, for example) and to how our bodies respond to it. Eventually, the changed virus might be able get through our immune response undetected. Such **antigenic drift** causes people who may have immunity from previous infections or vaccination to lose that immunity and be reinfected. The changes may also make the virus less susceptible to treatments or existing vaccines.

Antigenic drift is one of the reasons why influenza and COVID-19 vaccines must be reviewed and updated to keep up with the viruses as they change.

Focus Question

What are some other risks to the economy and/or society associated with having high numbers of people sick or quarantined? Consider all the types of products and services that are needed to keep a community functioning.

Prevention Strategies

To reduce the risk associated with a disease outbreak, the most suitable strategy depends on the specific chain of infection. Epidemiologists and public health officials usually focus on the segment in the infection chain that is most susceptible to intervention.

Epidemiologists also consider the length of time people are sick and how long previous outbreaks of similar diseases have lasted. For example, an outbreak may be connected to a single contamination of a food or water source. Mitigation in this case might involve identifying and eradicating the source of contamination. However, outbreaks of novel respiratory viruses can last for several years before they are contained.

Table 6 summarizes several common strategies that can be put into place at the individual or community level. Some can be applied either to all people or only to specific groups (for example, only to sick patients or those testing positive for the infection, or to large groups of people vs smaller groups).

Table 6. Common mitigation strategies for infectious diseases.

Strategy	Description
Isolation/quarantine	Physically isolate individuals who are infected (isolation) or who have been exposed (quarantine)
Travel restriction	Limit movement of an entire population, regardless of infection status
Social distancing	Reduce close contact in public settings
Closing schools	Move to online education, if possible
Canceling community events	Cancel concerts, sporting events, conferences, or other large assemblies of people
Spacing guidelines	Add guidelines to create space between people as they wait in line, sit at tables, etc.
Surface sanitization	Disinfect surfaces in areas where people congregate
Personal protective equipment (PPE)	Use clothing and other physical barriers to pathogen spread
Face mask or shield	Cover the nose and mouth or face; different degrees of filtration can be added with masks
Gown	Wear a disposable, sanitary gown to cover clothing
Gloves	Wear disposable, sanitary gloves to protect hands
Recalls	Stop sales of and ask consumers to return items that may be contaminated with a pathogen
Air filtration and ventilation	Employ mechanisms of improving indoor air quality and purity: ventilation improves air flow, filtration improves purity
Environmental monitoring	Test environmental reservoirs for pathogen presence; for example, test air or wastewater samples for presence of a pathogen
Testing	Perform diagnostic tests on the public to diagnose or monitor for infection; can be used to clear people for attendance at mass gatherings, work, or school
Pharmaceutical interventions	Use and develop medications that can treat symptoms or prevent infection
Vaccination	Primes the immune system, reduces number of susceptible hosts
Medication (antivirals)	Treat symptoms, reduce replication of viruses, and use other mechanisms for treating disease
Vector control	Inhibit movement or activity of pathogen-containing insects, animals, or other hosts or reservoirs

Each strategy has its pros and cons and may not be 100% effective. For example, isolating a population may be effective at reducing spread, but it will have unintended consequences on the ability of society to function. Often, a layered approach (the use of more than one strategy) is best. This is particularly true in the case of a novel virus like PLS-CoV, where it may be difficult to predict the outcomes and efficacy of any one approach.

Part 2: Developing a Response Strategy for the AnyTown PLS Outbreak

In this activity, in your role as an AnyTown public health official, you must decide which measures, if any, you will put in place to slow the spread of disease and mitigate its effects on the healthcare system and society.

Focus Questions

PLS-CoV is a novel virus, and the first decision to make is whether AnyTown should introduce prevention measures to contain its spread. If you were the Public Health Director, what other information would you need to be able to make this decision?

- ***Information about PLS-CoV and/or PLS disease:***

- ***Information about the AnyTown community and surrounding region:***

List three different strategies the population of AnyTown could use to reduce their personal risk of PLS-CoV infection. Consider the nature of PLS-CoV, the mode of transmission you found in Activity 3 for PLS-CoV, and the prevention strategies in Table 6.

- ***Briefly explain how each strategy works to minimize risk.***

- ***Are there any drawbacks to each strategy that might prevent people from using them?***

List three different prevention methods you could implement at the community level.

- ***Briefly explain how each strategy works to minimize the spread of PLS.***

- ***What are some potential societal and economic impacts of each of the three strategies?***

Describe a way in which three of the personal and/or community-level strategies might be used together to maximize effectiveness.

How might your personal and community-wide mitigation proposals change because of the following new findings during a PLS-CoV outbreak or epidemic?

- ***New, additional, and significant mode of transmission — spread by fomites:***

- ***New treatment — a new medication becomes available that reduces symptoms and hospitalization rates:***

- ***Vaccine — a vaccine becomes available that reduces transmission and/or disease severity:***

Appendix A

Naming Viruses and the Diseases They Cause

The International Committee on Taxonomy of Viruses (ICTV) gives official names to novel viruses according to genetic structure. The World Health Organization (WHO), in its International Classification of Diseases (ICD), gives names to virus-caused diseases.

In the past, viruses and the diseases they caused were often named according to the regions in which they were first found (“Norwalk Virus”), according to animal source (“Swine Flu”), or other considerations. This inconsistent system was not only uninformative, but it also created unintended negative impacts on trade, travel, tourism, or animal welfare and sometimes caused offense to certain cultural, social, national, regional, professional, and ethnic groups.

The current method is more informative because it names viruses according to their closest genetic relatives, which can provide insights into their behavior. It also names diseases according to symptoms or other aspects that enable discussion on disease spread, transmission, transmissibility, severity, and treatment.

As a recent example, the ICTV named “severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)” because the virus is genetically related to the coronavirus (CoV) responsible for the SARS (severe acute respiratory syndrome) outbreak of 2003. While related, the two viruses are different. The WHO announced “COVID-19” as the name of this new disease on 11 February 2020, following guidelines previously developed with the World Organization for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO). COVID-19 is an acronym for “coronavirus disease of 2019”.

Glossary

Aerosols — fine spray of respiratory droplets produced by exhalation and consisting of saliva, mucus, and other matter from respiratory tract surfaces; generally smaller in size than droplets, aerosols spread farther and can remain suspended in the air for much longer than droplets; transmission through aerosols, therefore, is considered an indirect mode of transmission.

Agarose gel electrophoresis — technique or process by which charged molecules like DNA are separated in an electrical field (see Electrophoresis); an agarose gel matrix is used to slow the movement of larger molecules relative to smaller ones.

Airborne — mode of transmission involving aerosols; occurs when bacteria or viruses travel in droplet nuclei that have been aerosolized.

Antibody test — diagnostic test that uses antibodies to detect the presence of other antibodies in the patient's bloodstream (to indicate a previous infection, for example).

Antigen test — diagnostic test that uses antibodies to detect the presence of an antigen (a virus or other pathogen, for example); designed to detect an active infection.

Antigenic drift — accumulation of mutations in genes that encode surface proteins of viruses that the host's antibodies recognize; results in a new strain of virus not effectively inhibited by the antibodies that prevented infection by recognized strains and makes it easier for the virus to spread through a partially immune population.

Asymptomatic infection — infection that does not result in symptoms; an infected individual who does not show symptoms of the infection.

Capsid — protein coat that surrounds the genetic material of a virus.

Contagious diseases — infectious diseases that spread easily from person to person.

Containment — action of keeping something harmful under control or within limits.

Direct transmission — movement of an infectious agent like a virus between two hosts in a manner that involves direct contact between the hosts and/or reservoir.

Droplets — liquid respiratory emissions produced by exhalation and consisting of saliva, mucus, and other matter from respiratory tract surfaces; generally larger in size than aerosols, droplets do not remain suspended in air as long as aerosols; transmission through droplets is considered a direct mode of transmission.

DNA virus — virus with a DNA genome.

Electrophoresis — movement and separation of molecules in an electrical field; positively charged particles move toward negatively charged poles, and vice versa.

Envelope — in reference to virus structure, the lipid covering of certain groups of viruses.

Epidemic curve — statistical chart used in epidemiology to visualize the onset of a disease outbreak; also known as an epi curve or epidemiological curve.

Eradicate — destroy completely, get rid of, put an end to.

Fecal-oral route — route of transmission of a disease in which pathogens pass in fecal particles from one person to the mouth of another person.

Genome — the complete set of genetic information of an organism (or virus).

Genome sequencing — determination of the nucleotide sequence of a genome.

Indirect transmission — movement of an infectious agent like a virus between two hosts in a manner that involves indirect contact between the hosts and/or contact with an intermediate surface or vector.

Infectious agent — pathogen; any organism or agent that can produce disease (can include viruses, bacteria, fungi, protists, algae, and other organisms).

Isolation — separation of an infected individual from others.

Mitigation — reduction of something harmful or the reduction of its harmful effects.

Mode of transmission — in epidemiology, the mechanism by which a pathogen like a virus moves from one host (or reservoir) to another.

Mutate — undergo a change in the genetic code; such a change may or may not result in a physical change.

Nasopharyngeal swab — method of collecting a sample of nasal secretions from the back of the nose and throat.

Nucleocapsid — capsid (protein coat) of a virus with the enclosed nucleic acid.

Pathophysiology — process by which an infection (for example, by a virus, bacterium, fungus, etc.) causes disease.

PCR test — polymerase chain reaction; diagnostic test designed to detect the presence of DNA by amplification of that DNA by PCR.

Portal of entry —path through which an infectious agent like a virus enters its host.

Portal of exit — path through which an infectious agent like a virus leaves its host.

Quarantine — separation of a potentially infectious person from the general population.

Reservoir — in epidemiology, refers to the habitat in which the infectious agent normally exists.

Respiratory-aerosol route — route of transmission of a disease in which pathogens pass from one person in respiratory droplets or aerosols to the mouth or nose another person.

Respiratory droplet — small aqueous excretion produced by exhalation and consisting of saliva, mucus, and other matter from respiratory tract surfaces; generally larger in size than aerosols, droplets tend to fall out of the air relatively quickly and so do not spread farther than a few meters; transmission through droplets, therefore, is considered direct transmission.

Reverse-transcription PCR (RT-PCR) test — diagnostic test designed to detect the presence of RNA; requires a reverse transcription step to convert the RNA to DNA before amplification by PCR.

RNA virus — virus with an RNA genome.

Susceptible host — host organism that is vulnerable to infection.

Symptomatic infection — infection that results in symptoms; an infected individual that shows symptoms of the infection.

Virion — viral particle that exists independently of a host cell; consists of the genome, protein capsid, and in some cases, lipid envelope.

Virology — subfield of microbiology, the study of viruses and their structure, classification, evolution, mechanisms of infection and reproduction, pathophysiology, and detection.

Zoonotic — viruses, bacteria, fungi, and other microbes that can spread from animals to cause disease in humans.

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