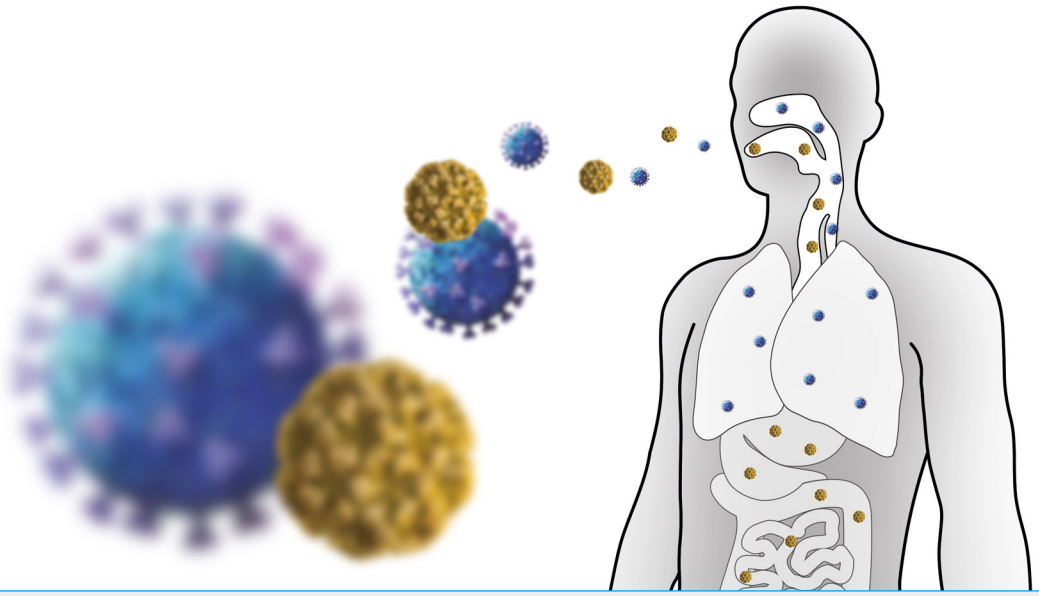
BIO-RAD Explorer



Virus Detection and Transmission Kit

(Norovirus)

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 Gastrointestinal Disease

A new gastrointestinal disease has been reported recently in the region surrounding AnyTown, USA. Called Extrusive Bowel Syndrome[1] (EBS), this disease is spreading rapidly and causes symptoms that include vomiting, nausea, stomach cramps, diarrhea, body aches, and fever. EBS has led to numerous hospitalizations, and patients are not recovering as quickly as they do from more common gastrointestinal infections.

Scientists have determined that EBS is caused by a novel norovirus (NoV) they call EBS-NoV. Noroviruses are a family of viruses that can cause gastroenteritis, or inflammation of the stomach and intestines. This leads to fast onset of nausea, vomiting, stomach cramps, and diarrhea. EBS-NoV is considered a "novel" norovirus because its **genome** is quite different from all other known strains.

Because EBS-NoV is novel, little is known about its source, how it spreads, how it causes disease, or how it should be treated. There is no vaccine or immunity within the population, so everyone is susceptible to infection.

As EBS-NoV spreads, doctors, researchers, and clinicians are working as quickly as possible to understand its **pathophysiology** and to find the most effective treatments. Molecular biologists have used the EBS-NoV **genome sequence** to develop a diagnostic test that can detect EBS-NoV 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 **source** and **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 EBS
- When one of the patients tests positive for EBS-NoV infection, you will then move into the role of a medical lab scientist and perform an EBS-NoV diagnostic test on samples collected from other suspected cases
- Finally, 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 EBS at a local restaurant

[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 Extrusive Bowel Syndrome (EBS) (Figure 1).

- Patient A, a 17-year-old female, is experiencing stomach cramps, diarrhea, nausea, vomiting, and a headache
- Patient B, a 32-year-old female, is experiencing nausea and vomiting, as well as some fatigue and dizziness

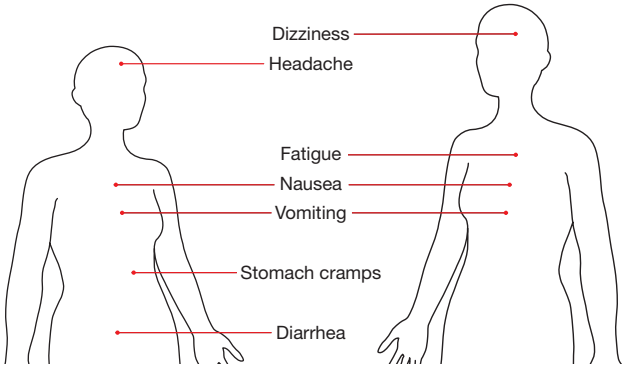


Figure 1. Patients A (left) and B (right).

As the ER physician, you examine both patients and find they have different symptoms that are characteristic of dehydration brought on by vomiting or diarrhea:

- Where a normal blood pressure reading for a 17-year-old is around 120/80 mm Hg, Patient A is exhibiting a low blood pressure of 98/65 mm Hg
- Patient B is experiencing dizziness, and where women of her age have an average heart rate of 69–75 bpm, she has an elevated heart rate of 92 bpm

You are aware of the novel virus that is causing gastrointestinal disease, but there are also other bacteria and viruses that cause food poisoning or gastrointestinal disease. Could it be the novel EBS-NoV? Or are these patients experiencing an infection by another virus or bacterium? Or could their symptoms indicate something else?

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

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

Symptoms	**EBS** (Extrusive Bowel Syndrome)	**Norovirus** (Genogroup I)	**Salmonella**	**Hyperemesis Gravidarum** (pregnancy-induced vomiting)
Nausea	Common	Common	Rare	Common
Vomiting	Common	Common	Rare	Common
Stomach cramps	Common	Common	Common	Sometimes
Diarrhea	Sometimes	Common	Common	Sometimes
Bloody diarrhea	Sometimes	Rare	Sometimes	Never
Fever	Sometimes	Sometimes	Common	Never
Muscle or body aches	Sometimes	Sometimes	Sometimes	Never
Headache	Sometimes	Sometimes	Sometimes	Sometimes
Dizziness	Common	Common	Common	Common
High heart rate	Rare	Sometimes	Common	Common
Low blood pressure	Common	Common	Common	Common
Time to onset	12–24 hours	12–48 hours	6 hours–6 days	4–6 weeks of pregnancy
Tests available				
Diagnostic	Yes	Yes	Yes	Yes
Treatments available	Unknown, patient **isolation** recommended	Rehydrating drinks; patient isolation recommended	Rehydrating drinks, antibiotics	Fluids and anti-nausea medicine

Focus Questions

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

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

EBS is a new 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.

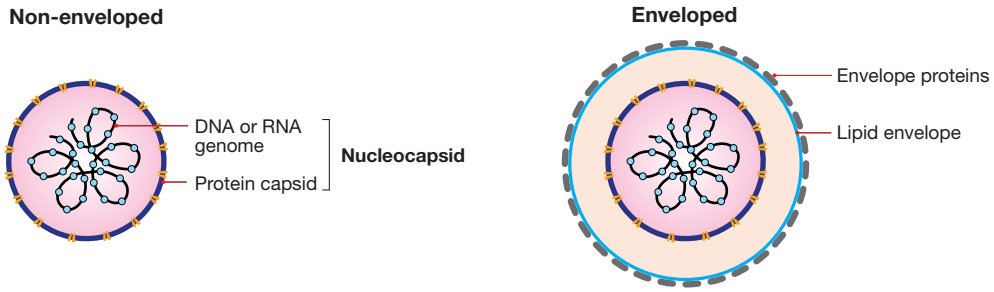


Figure 2. Structure of a virion. Non-enveloped viruses (left) do not have the lipid membrane that enveloped viruses (right) have.

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

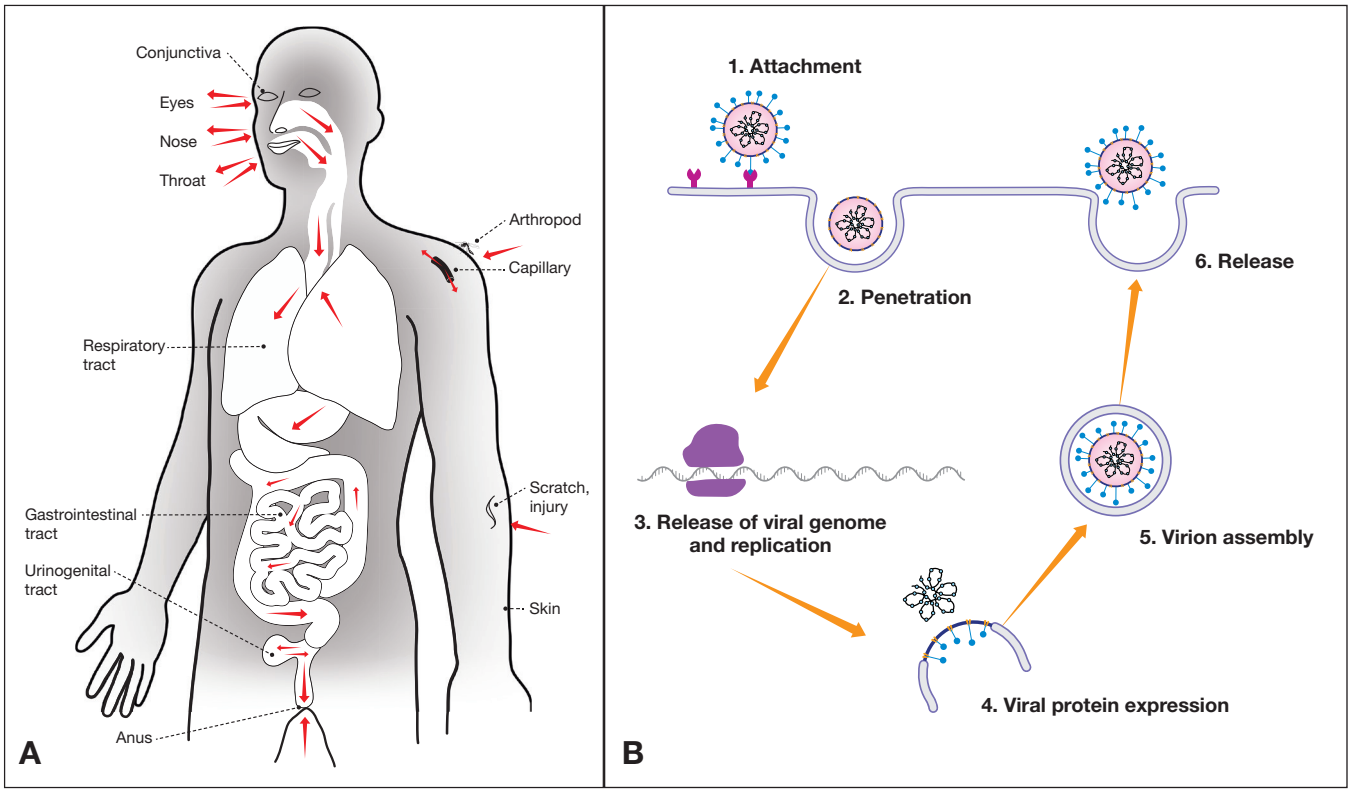


Figure 3. Portals of entry into a human host (A) and the stages of viral infection of a host cell (B).

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 norovirus 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.

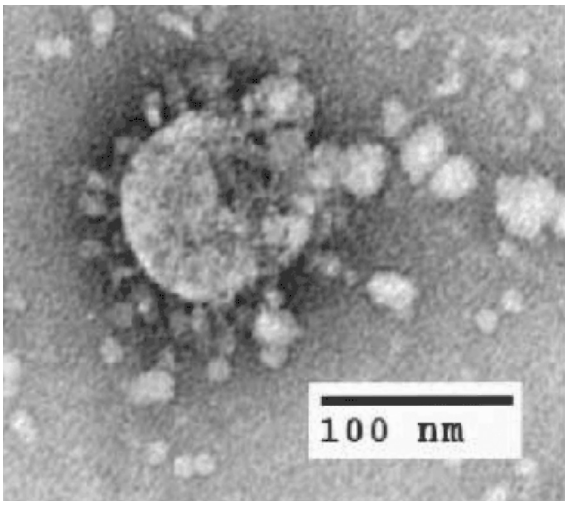


Figure 4. SARS-CoV particle. Negative stain electron microscopy of a SARS-CoV virion. Note the characteristic club-shaped projections surrounding the periphery of the virion. Source: C.D. Humphrey, CDC (https://www.cdc.gov/sars/lab/images.html).

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, 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.

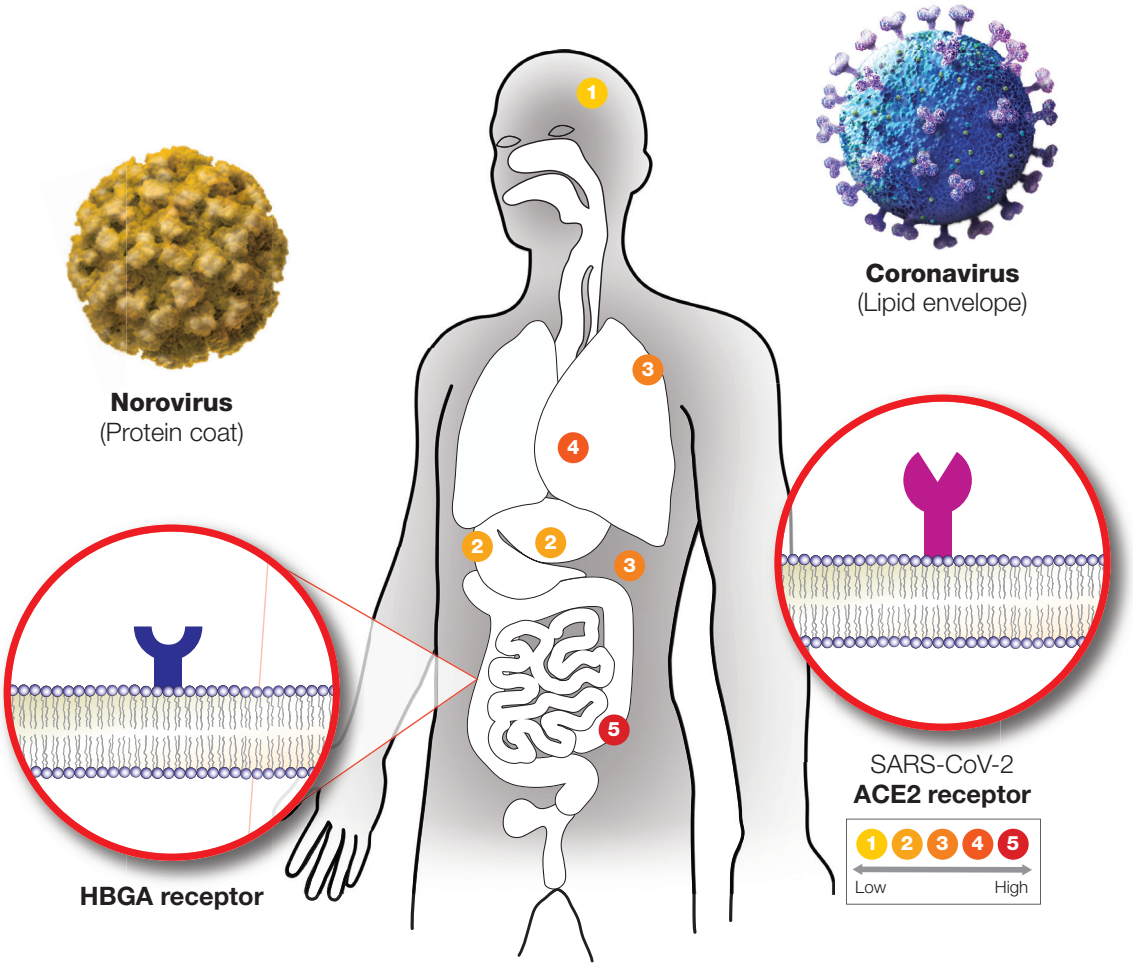


Figure 5. Viral pathogenesis. Different viruses target different tissues based upon receptor preference. Noroviruses bind to HBGA receptors, which occur primarily in the gastrointestinal tract. The SARS-CoV-2 virus binds to ACE2 receptors, which are found in various tissues and organs throughout the 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 an 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)

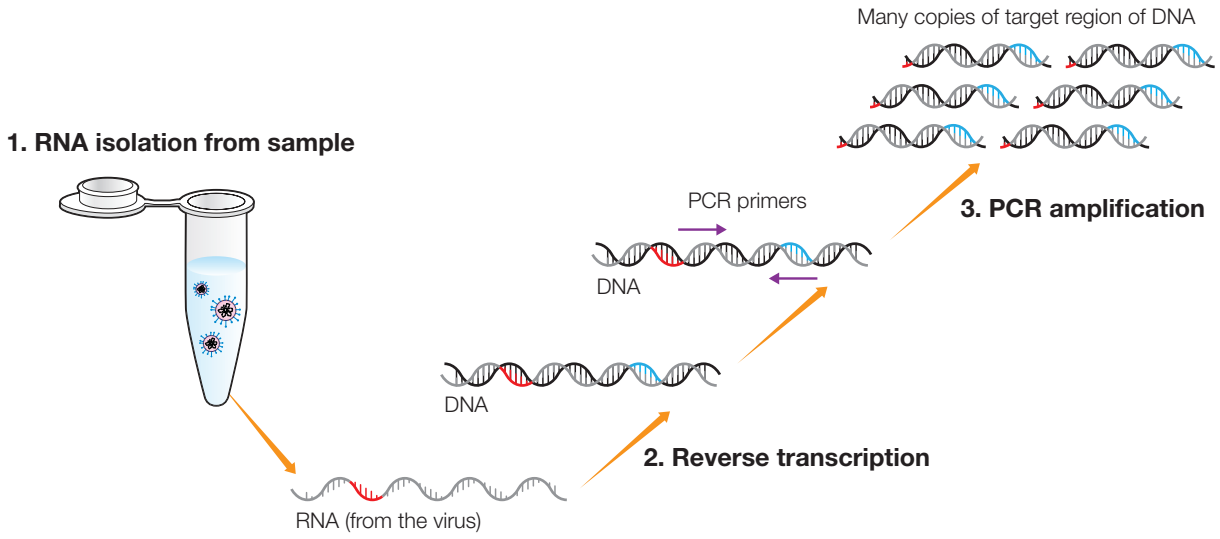


Figure 6. Reverse-transcription PCR (RT-PCR). RT-PCR uses an enzyme called reverse transcriptase to reverse the transcription process and convert RNA back into DNA. The DNA then serves as the template in a PCR. In this diagram, the purple arrows on the DNA represent primers, short sequences of DNA that 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 both Patient A and Patient B may be infected with a norovirus or *Salmonella*, or they may be pregnant. You order diagnostic tests for all these conditions, but because you know EBS-NoV is circulating in the region, you also order a newly developed PCR-based test for EBS-NoV.

About the EBS-NoV Test

The AnyTown Hospital has access to a new **RT-PCR** test that can detect the presence of EBS-NoV in human samples.

NoVs are gastrointestinal viruses, and they are most easily detected in the stool or vomit of infected people. For this test, the lab technician collects stool samples from Patients A and B using toilet hats (Figure 7). Toilet hats are plastic bowls that sit underneath toilet seats to easily collect stool from patients. The lab technician then uses a small scoop to take a sample of each patient's stool.

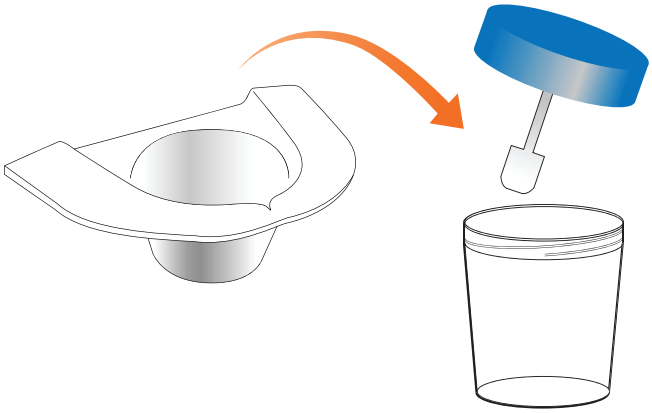


Figure 7. Collection of stool sample from a toilet hat.

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

- **A gene specific to the virus EBS-NoV** — the test detects the *VP1* gene, which encodes the capsid protein of EBS-NoV. The test is designed to detect only a specific section of the *VP1* gene of EBS-NoV. It will not detect *VP1* genes from any other NoVs 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 EBS-NoV diagnostic test.

Target region	Band size	Purpose
EBS-NoV *VP1* gene	~530 bp	Detects presence of EBS-NoV
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 *GAPDH*, and one set will bind to and amplify the *VP1* 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.

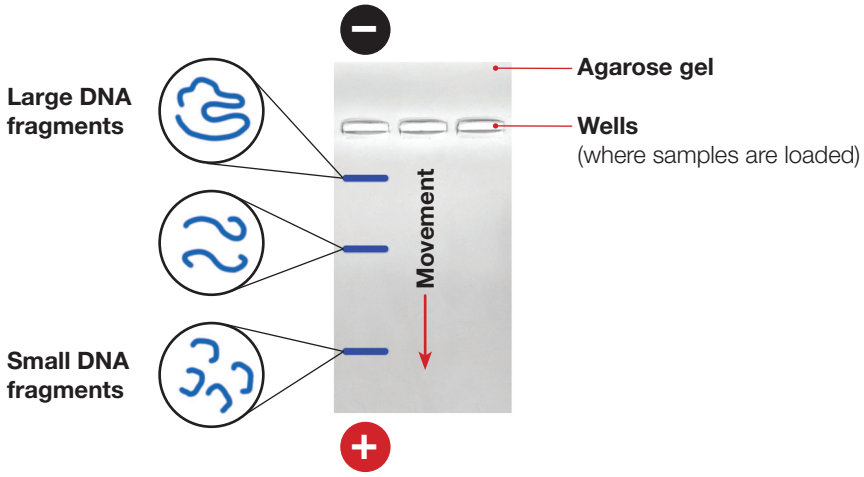


Figure 8. Agarose gel electrophoresis. By convention, the rectangular spaces across the top of a gel represent the wells into which samples are loaded. The negatively charged DNA fragments migrate through the gel toward the positive pole (anode) when a voltage is applied. The straight path the DNA fragments 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 EBS-NoV 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 will move farther through the maze in each period than larger chains.

The stool sample contains any infecting virus as well as some human cells that have sloughed off during sample collection. In this EBS-NoV test if the RNA sample was successfully collected, extracted, and amplified:

- All human patient samples should have a band for *GAPDH* gene
- Only patients infected with EBS-NoV will also have the *VP1* gene band, which is specific to EBS-NoV

Focus Questions

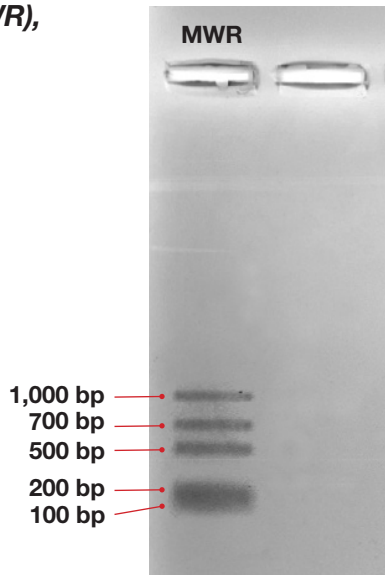
In the EBS-NoV test, which target region — the VP1 gene or GAPDH — would produce a band that would move farther than the other by agarose gel electrophoresis? Why?

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

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 EBS-NoV tests for:

- ***The amplified VP1 gene target***
- ***The amplified GAPDH gene target***



Patient Data

Now that you understand how the EBS-NoV 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 EBS-NoV
- **(+) Control** — this control has both the *VP1* and the *GAPDH* genes; it simulates a sample from a person who is infected with EBS-NoV
- **NTC (no-template control)** — this control is a sample that has only the PCR primers and no DNA template
- **Patient A**
- **Patient B**

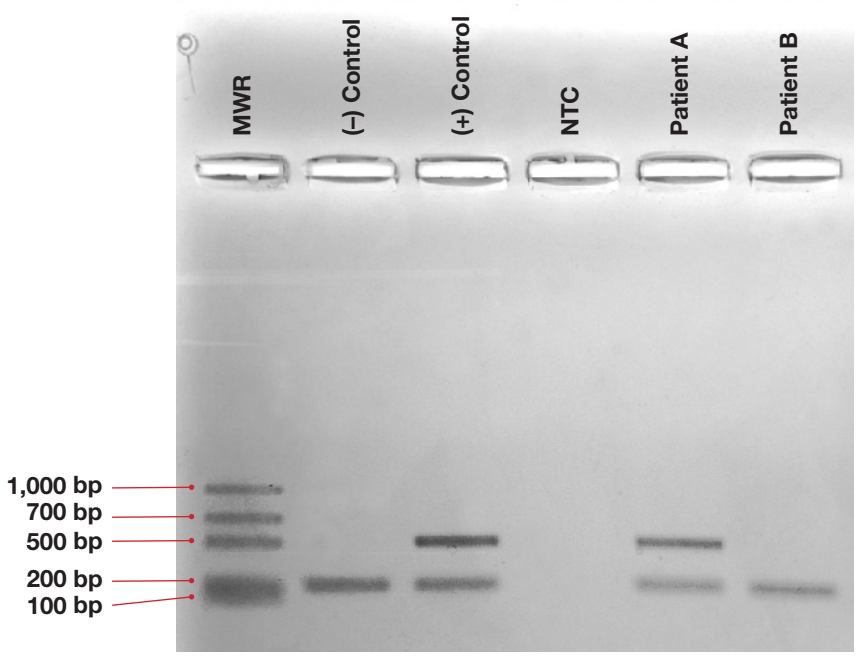


Figure 9. Agarose gel electrophoresis of the controls and patient samples from the EBS-NoV diagnostic test. This gel shows the bands obtained from an RT-PCR test, including primers that amplify the EBS-NoV *VP1* gene and the human *GAPDH* gene. MWR = molecular weight ruler; (–) control = control sample containing the human *GAPDH* gene; (+) = positive control sample containing both the *VP1* 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 EBS-NoV infection to local public health officials (upon additional testing, Patient B was found to be pregnant and sent home with anti-nausea medication).

The next day, the officials took a detailed patient history for Patient A and, to contain the spread of EBS-NoV, they initiated contact tracing to notify all known contacts of Patient A of their exposure and ask them to be tested.

Before she showed up with symptoms at the AnyTown Hospital in the late evening of June 8, Patient A had been at the AnyTown Restaurant for the lunch service. At the time, she was experiencing only mild nausea and diarrhea. But by evening, her symptoms had worsened, and she was in the ER. The public health officials then 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 these people and learned that a significant number of them were experiencing symptoms like those of Patient A. All agreed to submit stool samples 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 NoV, EBS-NoV.

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 EBS-NoV diagnostic test.

Sample	Description	Purpose
MWR	Molecular weight ruler	
(–)	Control sample containing the *GAPDH* gene	
(+)	Control sample containing both the *VP1* and *GAPDH* genes	
NTC	No-template control (water; no DNA)	
Restaurant samples	Four staff/patron samples designated by a number	

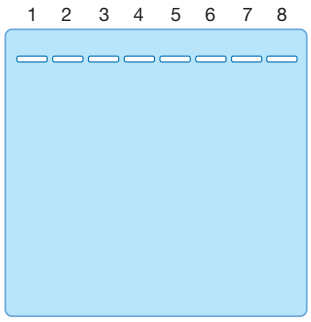
Part 2: Sample Analysis by Agarose Gel Electrophoresis

Student workstation	Quantity
Molecular weight ruler (MWR), 20 µl	1
GAPDH control (–), 10 µl	1
VP1 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

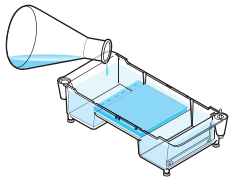
Running DNA on an Agarose Electrophoresis Gel

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



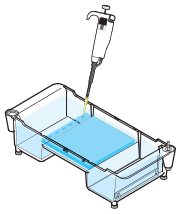
2. Place a 1% TAE agarose gel into the electrophoresis chamber. Be sure that the gel is oriented so that the wells are closest to the black (–) electrode, or cathode.

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

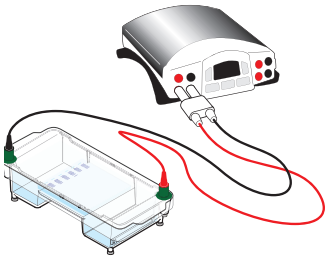


4. Using a fresh pipet tip for each sample, load 10 µl of each DNA sample and 20 µl of MWR into each gel according to the table below.

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



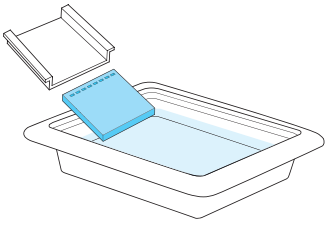
5. Place the lid on the electrophoresis chamber and connect the electrical leads to the power supply, red to red and black to black.



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 EBS-NoV 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 EBS, 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 EBS-NoV 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

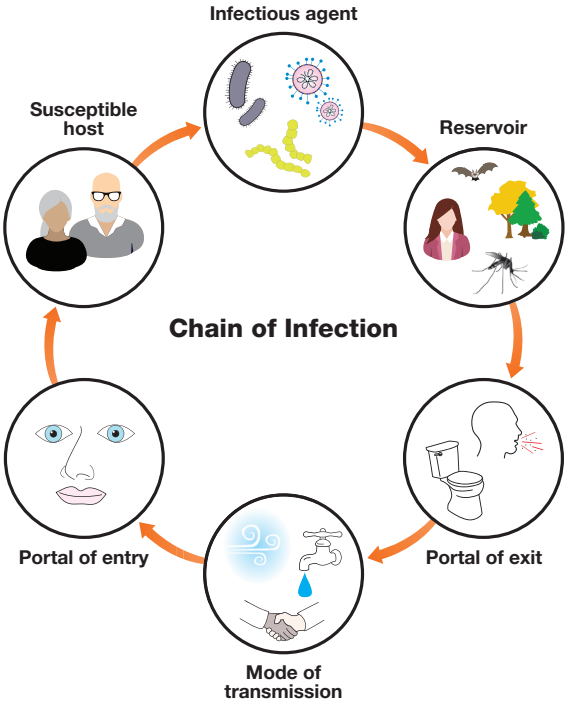


Figure 10. Chain of infection. In epidemiology, a chain of infection has six main points.

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, EBS-NoV) 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.

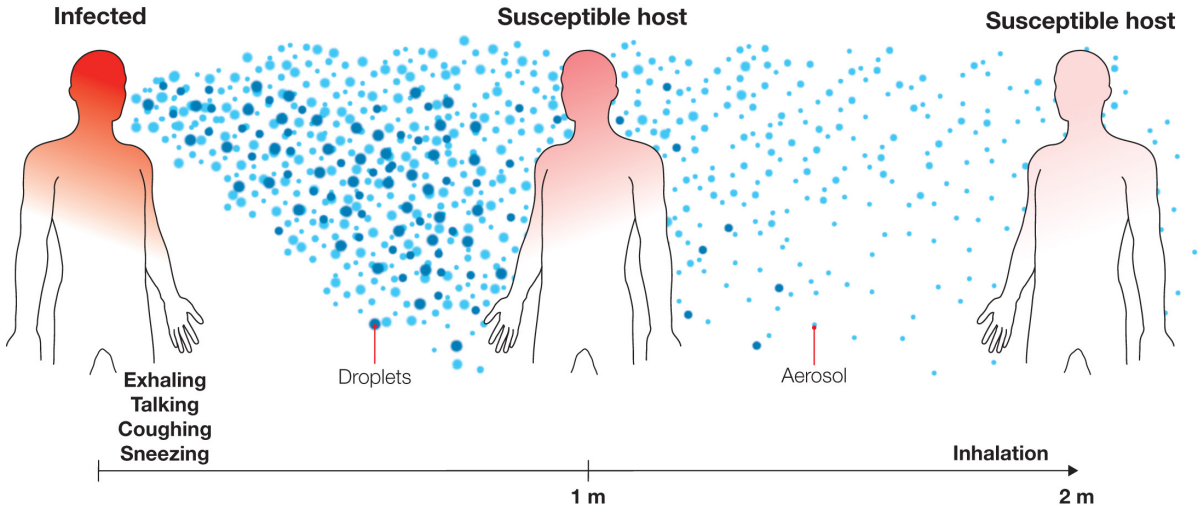


Figure 11. Droplet and airborne transmission. When an infected person exhales, talks, coughs, or sneezes, virus-laden drops are transported through the air, where a potential host may inhale them to initiate a new infection. The smaller the droplet, the farther it may drift. Larger droplets (>5 µm) tend 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 non-respiratory 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 EBS-NoV 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 EBS-NoV at AnyTown restaurant. Closely examine the restaurant layout, seating chart, patient movement, and other available data. Remember EBS-NoV is a novel coronavirus and so may have a different mode of transmission from other NoVs.***
3. ***Explain how the evidence supports your proposal.***
4. ***Build a model to propose how EBS-NoV 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?***

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 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.

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.

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.

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.

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 sequence — 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; germ; can include viruses, bacteria, fungi, protists, algae, and other organisms.

Isolation — separation of an infected individual from others.

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 to the organism.

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

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

PCR test — 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.

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 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 who 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.

References

Gralton J et al. (2011). The role of particle size in aerosolised pathogen transmission: A review. J Infect 62, 1–13.

Nicas M et al. (2005). Toward understanding the risk of secondary airborne infection: emission of respirable pathogens. J Occup Environ Hyg 2, 143-54.

Siegel J et al. (2007). Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Health Care Settings. Am J Infect Control 35, S65-164.

Tellier R (2009). Aerosol transmission of influenza A virus: a review of new studies. J R Soc Interface Suppl 6, S783-90.

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