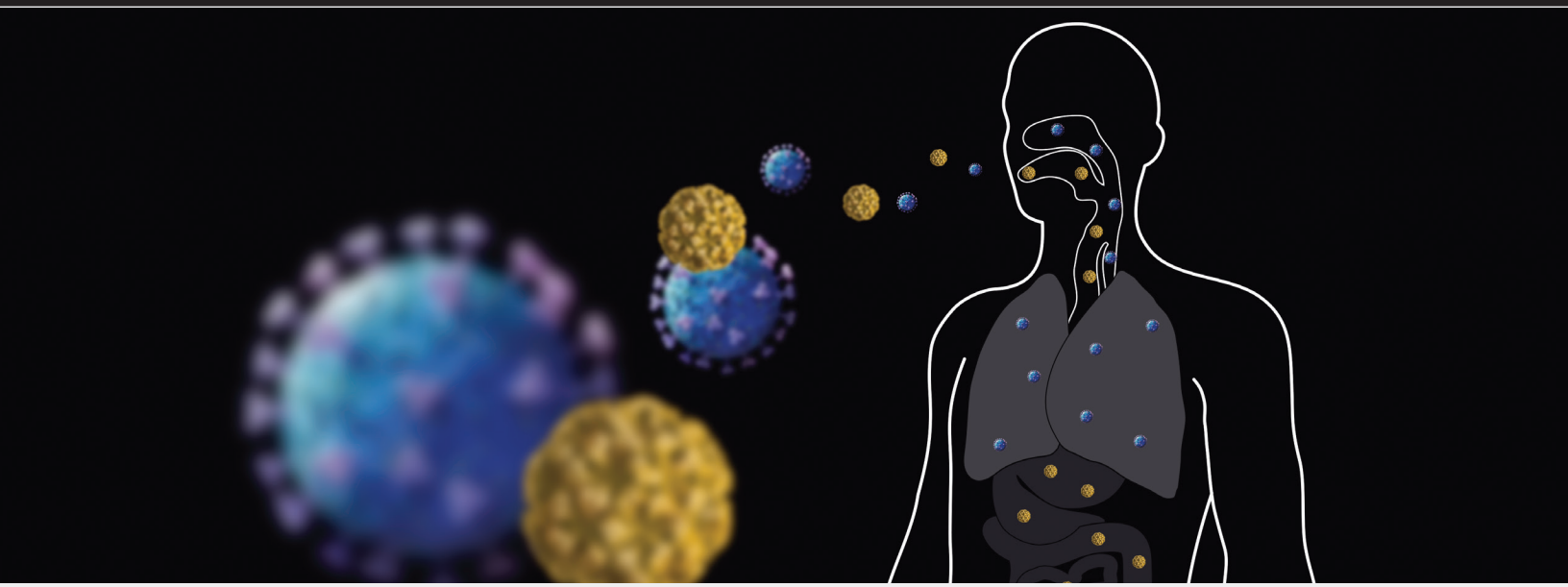
BIO-RAD Explorer



Virus Detection and Transmission Kit

Catalog #17008261EDU

Instructor 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

Dear Instructor

Thank you for fostering curiosity in our future scientists and citizens as they prepare for a future of thinking critically, applying knowledge, asking questions, and working collaboratively to solve real-world problems.

In 2020, the world changed for you and for your students as the COVID-19 pandemic spread across the globe and into your community. As scientists worked quickly to identify the virus responsible and create tests and treatments, public health officials worked hard to decide the best course of action to keep communities safe. The response to any pandemic is an intricate and complex dance between established science, constantly evolving data, and the desire to keep people safe without unforeseen negative impacts on economies, social safety nets, and overall public health.

The Virus Detection and Transmission Kit teaches the core skill of DNA gel electrophoresis within a context that gives students a broad understanding of virology, viral pathology, epidemiology, and an application of PCR. It also offers insights into a range of professions that intersect and work together during the response to a viral disease outbreak. Students begin by working through a fictional patient diagnosis and then learn how PCR can be used in pathogen detection. They use agarose gel electrophoresis on simulated DNA samples to determine who in a restaurant was infected by a novel virus. Students then apply their data to other pieces of information to construct an evidence-based explanation for how the virus was transmitted throughout the restaurant.

This kit offers you a choice of four different scenarios: two scenarios for a coronavirus, and two scenarios for a norovirus. The coronavirus activities include an optional activity in which students explore the considerations public health officials must make in response to an outbreak of a novel virus. It is our hope you will find the scenario that best engages your students and supports your curriculum.

We strive to continually improve our curricula and products, and your input is extremely important to us. We welcome your stories, comments, and suggestions.

Bio-Rad Explorer Team
Bio-Rad Laboratories
6000 James Watson Drive, Hercules, CA 94547
explorer@bio-rad.com

Contents

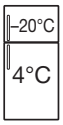
Kit Storage

When you receive the Virus Detection and Transmission Kit:

1. Record storage location and batch numbers from the product labels.

2. Store the **DNA Electrophoresis Samples bag** in the freezer(–20°C).

   For short-term storage, the **reagent bag** may be stored in the refrigerator (4°C) for up to 1 month.



3. If using **UView 6x Loading Dye and Stain**, store it in the refrigerator (4°C).

4. Store the **remaining reagents and consumables** at room temperature (~20°C).

5. Review this **Instructor Guide** and included **Answer Guide** and choose which of the four scenarios to run with your class.

6. Visit **bio-rad.com/VDTkit** to download the **Student Guide** for your choice of activity (coronavirus or norovirus).

Technical Support is available at support@bio-rad.com or 1-800-4BIORAD, option 2.

Safety Guidelines

Always follow basic laboratory safety guidelines: wear gloves and safety goggles; do not eat, drink, smoke, or apply cosmetics in the work area. If any solution gets into a student's eyes, flush with water for 15 minutes.

Use special caution when working with hot molten agar while preparing gels: wear heat-resistant gloves, goggles, and a laboratory coat.

If using Fast Blast DNA Stain, note it is not toxic but will stain hands and clothes. Wear gloves and lab coats to prevent stains.

If using UView Loading Dye and Stain, use appropriate personal protective equipment when using a transilluminator, as UV light can irradiate skin and eyes.

Considerations for Sensitive and Complex Subject Matters

Some of your students may have personal connections to the issues around the COVID-19 pandemic. They may know someone affected or they may harbor misconceptions about the causes of illness or strategies undertaken to limit infection (for example, lockdowns, mask mandates, and vaccination). This kit offers a choice of focusing on a norovirus or coronavirus spread, so choose the activity that you feel might be most appropriate for your classroom.

Before addressing this sensitive topic in the classroom, you may want to prepare yourself to provide additional support for your students. Appendix D has some resources to assist.

Students likely have differing opinions on how the COVID-19 pandemic was addressed, and the discussions your students may have while going through this set of activities provide opportunities to discuss medical privacy, the consequences of unlimited virus spread, the unintended consequences of different mitigation measures, and the important and varied contributions of various areas of expertise. Be sure students understand that these topics are not straightforward, and that research and policies are in constant development and must change when new information becomes available.

Kit Components

Each kit contains materials for eight (8) student workstations.

Virus Detection and Transmission Kit	**Quantity**
1.5 ml EZ Micro Test Tubes	90
Molecular Weight Ruler	200 µl
DNA Sample 1	215 µl
DNA Sample 2	250 µl
Orange G Loading Dye, 5x	1 ml

Small Fast Blast DNA Electrophoresis Pack	**Quantity**
Fast Blast DNA Stain	100 ml
Certified Molecular Biology Agarose	5 g
TAE Electrophoresis Buffer, 50x	100 ml

Small UView DNA Electrophoresis Pack	**Quantity**
UView 6x Loading Dye and Stain	200 µl
Certified Molecular Biology Agarose	5 g
TAE Electrophoresis Buffer, 50x	100 ml

Required Materials (not included in this kit)	**Quantity**
20–200 µl adjustable-volume micropipet and tips	1
2–20 µl adjustable-volume micropipet and tips	8
or 10 µl fixed-volume micropipet and tips	8
Microcentrifuge tube rack	8
Horizontal gel electrophoresis chamber	4–8
Power supply	1–8
UV transilluminator (if using UView 6x Loading Dye and Stain)	1
Marking pen	8
Waste container	8
Gel staining tray	8

Refer to Appendix A Ordering Information for ordering details for some of these required materials and for kit refills.

Microcentrifuge tubes, molecular weight ruler, DNA Sample 1, DNA Sample 2, and Orange G Loading Dye, 5x

Fast Blast DNA Stain, Certified Molecular Biology Agarose, and TAE Electrophoresis Buffer, 50x

UView 6x Loading Dye and Stain, Certified Molecular Biology Agarose, and TAE Electrophoresis Buffer, 50x

Introduction

Solve the Mystery of How a Virus Spread through a Restaurant

Two patients stumble into a hospital with symptoms associated with a newly emerging virus. Doctors diagnose the patients and find one is infected with the novel virus. This person had eaten lunch at a local restaurant earlier that day, so public health officials track down others who were also at the restaurant to determine whether and how the virus may have spread. Based on published viral transmission case studies, this hands-on lab activity puts your students into the roles of an emergency room (ER) doctor, medical laboratory scientist, epidemiologist, and public health official as they analyze patient samples and use patient information to determine how a novel virus strain spread through a restaurant.

In this laboratory activity, students analyze simulated samples collected from staff and customers at a fictitious restaurant where a viral transmission event took place.

- Use one of four different viral transmission scenarios: choose between two different viruses, each with two different transmission scenarios
- The patterns of infection among the customers in the restaurant vary between the scenarios to highlight different aspects of viral disease transmission
- See the Preparation Instructions section in this guide for details of the four different scenarios
- Select a scenario for your class before preparing reagents

Your students compile classroom sample data and propose explanations for how the virus was transmitted.

Resources

Student Guides

There are two Student Guides:

- Student Guide (CoV) covers your choice of two coronavirus transmission scenarios
- Student Guide (NoV) covers your choice of two norovirus transmission scenarios

Choose which virus and transmission scenario to run with your class. Each guide has the background information you and your students will need. This Instructor Guide highlights key points to emphasize with your students and provides additional details to help you address questions that may come up during class discussion.

Answer Guide

The Answer Guide provides extra details about the transmission scenarios that should be withheld from students to allow them to get the most out of the activity. Review the details in the Answer Guide about each transmission scenario to help you select the one that would be best for your classroom.

Other Resources

Please refer to Appendix D Resources for additional information and instructional media.

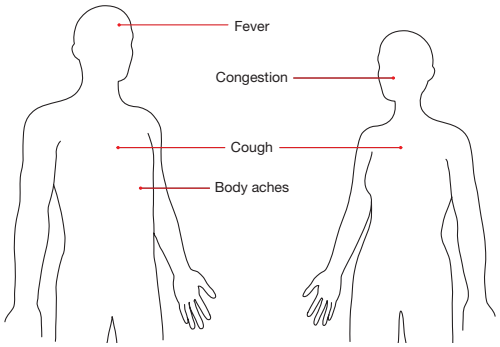
Kit Activity Overview

The Virus Detection and Transmission Kit allows you to conduct your choice of four different virus transmission scenarios: two different transmission scenarios for each of two different viruses (coronavirus or norovirus).

Activity 1

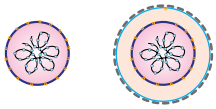
Learning about Virus Biology and Virus Detection

Two patients come to the emergency room at AnyTown Hospital. Both are showing symptoms of a viral disease. Students assess the symptoms and order a molecular test to assist them in their diagnosis.



Patient Symptom Review

Students evaluate the patients' symptoms against a list of known afflictions and make a hypothesis about possible causes.



Learning about Viruses, Pathophysiology, and Detection

Students read about and discuss viral biology, viral structure, and viral pathophysiology (how viruses cause disease). These concepts are important for understanding viral detection and modes of transmission.

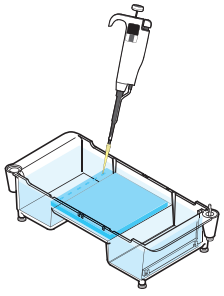
Patient Diagnosis

Students learn about molecular diagnostic tests in general, and about the components of a reverse-transcription polymerase chain reaction (RT-PCR)-based test and agarose gel electrophoresis. They analyze provided data to determine the infection status of the two patients.

Activity 2

Detecting Infections

Students analyze samples obtained from the staff and patrons of a local restaurant where the infected patient had been on the day of diagnosis.



Sample Analysis by Agarose Gel Electrophoresis

Students perform agarose gel electrophoresis on staff and patron samples and controls.

Collecting Class Data

Students combine their data into an analysis of the infection status of the entire restaurant.

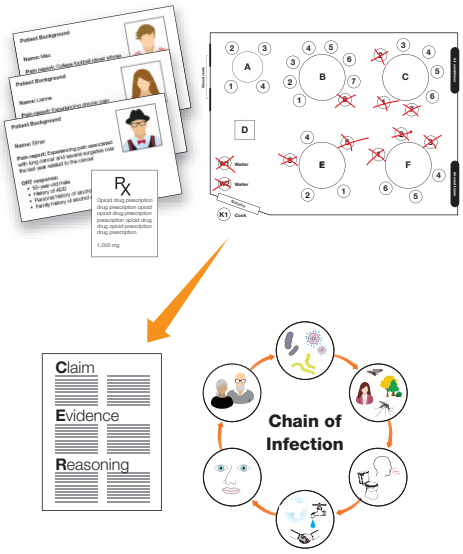
Activity 3

Building Transmission Models

Students integrate the diagnostic test data with other information about the restaurant patrons and staff to figure out how the virus spread throughout the restaurant.

Understanding the Chain of Infection
Students learn the six components of a chain of infection.

Creating a Transmission Model
Students analyze the complete set of data from the restaurant and additional restaurant information provided to propose an explanation for how the virus spread.



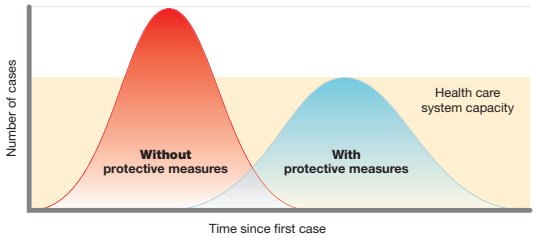
Activity 4

Mitigating Risk (CoV Activities, optional)

Students complete their investigation by considering whether and how to address the outbreak.

Understanding Response Strategies to Disease Outbreaks
Students learn how public health officials develop strategies for mitigating disease outbreaks in communities.

Developing a Response Strategy for the AnyTown Outbreak
Students develop their own strategies for mitigating spread of the virus in question.



Activity Timelines

The activities in this kit are designed to take four 45-minute class periods as shown in Table 1.

Table 1. Alternative timelines.

Class Period 1	Class Period 2	Class Period 3	Class Period 4 (CoV Activities, Optional)
In-class or out-of-class	In-class	In-class	In-class or out-of-class
Activity 1 Part 1. Patient Symptom Review Part 2. Learning about Viruses, Pathophysiology, and Detection Part 3. Patient Diagnosis	**Activity 2** Part 1. Pre-Laboratory Questions Part 2. Sample Analysis by Agarose Gel Electrophoresis Part 3. Analysis Part 4. Collecting Class Data[1]	**Activity 3** Part 1. Understanding the Chain of Infection Part 2. Creating a Transmission Model	**Activity 4** Part 1. Understanding Response Strategies to Disease Outbreaks

Curriculum Fit

Required Prior Knowledge

- Cell structure and basic cell theory
- Basic DNA, RNA, lipid, and protein structure and function
- Roles of DNA and RNA in encoding heritable information
- Basic principles of PCR and DNA electrophoresis
- How to use a micropipet

Curriculum Fit and Topic Considerations

- **Biology and virology** — students explore concepts surrounding basic virus structure and pathophysiology, including the function and interaction of viral proteins, lipids, and nucleic acids
- **Human body systems** — the two viruses that are the focus of this investigation infect the human respiratory and gastrointestinal systems, which are also reviewed
- **Careers in the life sciences** — throughout the activities, career highlights expose students to various career options and paths
- **Science practices** — in these activities, students analyze data, incorporate knowledge of virology to deduce a mode of transmission in a restaurant
- **Ethical, legal, and social issues** — there are broader issues associated with viral outbreaks and a community response, including health, social, and economic impacts

[1] Depending on the gel staining method used, this step may move to class period 3.

Preparation Instructions

Preparation Step	Time Required	When to Begin Preparation
Select the virus and transmission scenario	5–15 minutes (reading)	Prior to reagent preparation
Prepare reagent stocks	30 minutes	Up to a year before Activity 2 (store the stocks in the refrigerator for one month or at –20°C for up to one year)
Aliquot controls and samples	30 minutes	Up to a year before Activity 2 (store the controls and samples in the refrigerator for one month or at –20°C for up to one year)
Prepare agarose gels, TAE buffer, and stain	1 hour	Up to a week before Activity 2 (store gels in a sealable plastic bag with a few ml of buffer at room temperature for up to 1 day, or in the refrigerator for up to 1 week; store TAE buffer and Fast Blast Stain at room temperature)

Select the Virus and the Transmission Scenario

First, select one of the virus and transmission scenarios described below. Then:

- Print or download and review the Student Guide that corresponds to the virus you will be investigating
- Label and dispense DNA samples using the table corresponding to the scenario you chose

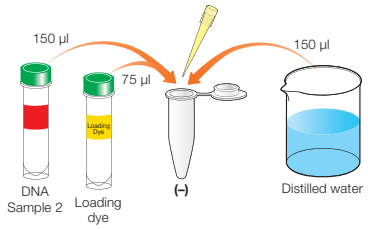
The mode of transmission impacts the distribution of positive and negative test results in the restaurant. **Select the virus and mode of transmission before preparing the samples.** Refer to the Answer Guide for more details on the specific aspects of each scenario below.

Coronavirus (CoV)	Norovirus (NoV)
Analysis of transmission of a fictitious new strain of coronavirus named PLS-CoV, which causes a new fictitious respiratory disease called pneumonia-like syndrome, or PLS.	Analysis of transmission of a fictitious new strain of norovirus named EBS-NoV, which causes a new fictitious disease called Extrusive Bowel Syndrome, or EBS.
Scenario CoV 1 **(Indirect transmission through airborne aerosols)** This activity is based on a real event that occurred early in the COVID-19 pandemic (Lu et al. 2020). At the time of the spreading event, few details were known about how the virus spread. In this scenario, staff and patrons of a fictitious restaurant were primarily infected by inhalation of small, airborne droplets containing virus particles.	**Scenario NoV 1** **(Indirect transmission through contaminated food)** In this scenario, patients were primarily infected indirectly by contaminated food.
Scenario CoV 2 **(Direct transmission through airborne droplets)** In this scenario, restaurant staff and patrons were primarily infected by short-range, direct exposure to large droplets containing virus particles.	**Scenario NoV 2** **(Indirect transmission through contaminated food and fomites)** In this scenario, patients were primarily infected indirectly by fomites (contact with contaminated surfaces) and contaminated food.

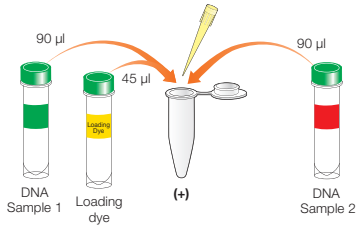
Prepare Reagent Stocks

Reagent stock preparation will take 30 min and may be done up to a year before Activity 2. Store the DNA sample stocks in the refrigerator for one month or at –20°C for up to one year.

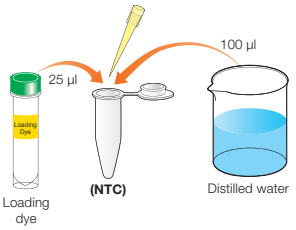
1. ***GAPDH* control stock (–).** Label a microtube (**–**). Using a fresh pipet tip each time, add the following and pipet to mix:
   - 150 µl DNA Sample 2
   - 150 µl distilled water
   - 75 µl loading dye (Orange G or UView 6x Loading Dye and Stain)



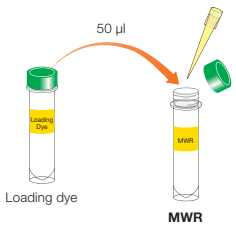
2. **Virus + *GAPDH* DNA stock (+).** Label a microtube (**+**). Using a fresh pipet tip each time, add the following and pipet to mix:
   - 90 µl DNA Sample 1
   - 90 µl DNA Sample 2
   - 45 µl loading dye (Orange G or UView 6x Loading Dye and Stain)



3. **No-template control (NTC).** Label a microtube (**NTC**). Using a fresh tip add the following and pipet to mix:
   - 100 µl distilled water
   - 25 µl loading dye (Orange G or UView 6x Loading Dye and Stain)



4. **Molecular weight ruler (MWR).** Using a fresh pipet tip, add 50 µl Orange G Loading Dye or UView 6x Loading Dye and Stain to the molecular weight ruler. Pipet to mix.



Aliquot Controls and Restaurant Staff and Patron Samples

Labeling and aliquoting tubes will take 30 min and may be done up to one year ahead of Activity 2. Store the DNA samples in the refrigerator for one month or at –20°C for up to one year.

Controls

5. Label tubes and fill them according to the following table.

Quantity	Tube Label	Sample Stock	Volume
8	**(–)**	**(–)**	11 µl
8	**(+)**	**(+)**	11 µl
8	**NTC**	**NTC**	11 µl
8	**MWR**	**MWR**	22 µl

Restaurant Samples

6. Choose the scenario you will be running with your class, then label 32 tubes as indicated in the following table.

   - **Omit** the tube corresponding to Patient A for your scenario (for example, for scenario NoV 1, omit E4)

7. Dispense 11 µl of each sample stock into each tube according to the table.

   - **Use only the column that matches your chosen scenario.** For example, for scenario NoV 1, tube A4, add 11 µl of (–) sample stock

Seat	CoV Scenario	
	CoV 1	CoV 2
A1	(–)	(–)
A2	(+)	(–)
A3	(+)	(–)
A4	(+)	(–)
B1	(+)	(–)
B2	(+)	(–)
B3	(Patient A)	(–)
B4	(–)	(–)
B5	(–)	(–)
B6	(+)	(–)
B7	(–)	(–)
B8	(+)	(+)
C1	(–)	(+)
C2	(–)	(–)
C3	(+)	(–)
C4	(–)	(–)
C5	(–)	(–)
C6	(+)	(–)
C7	(–)	(+)
E1	(–)	(–)
E2	(–)	(–)
E3	(–)	(+)
E4	(–)	(+)
E5	(–)	(–)
F1	(–)	(+)
F2	(–)	(+)
F3	(–)	(+)
F4	(–)	(–)
F5	(–)	(–)
F6	(–)	(–)
W1	(–)	(Patient A)
W2	(–)	(+)
K1	(–)	(–)

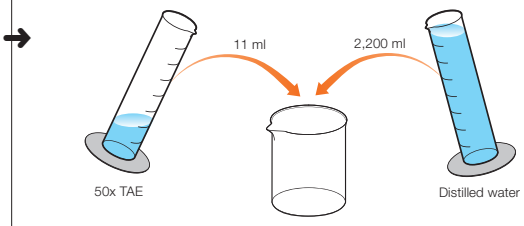
Seat	NoV Scenario	
	NoV 1	NoV 2
A1	(–)	(–)
A2	(–)	(+)
A3	(+)	(–)
A4	(–)	(–)
B1	(–)	(+)
B2	(+)	(–)
B3	(+)	(–)
B4	(–)	(–)
B5	(–)	(–)
B6	(–)	(+)
B7	(+)	(–)
B8	(–)	(–)
C1	(–)	(–)
C2	(–)	(–)
C3	(–)	(–)
C4	(+)	(–)
C5	(–)	(–)
C6	(–)	(–)
C7	(+)	(+)
E1	(–)	(–)
E2	(–)	(+)
E3	(–)	(–)
E4	(Patient A)	(–)
E5	(–)	(–)
F1	(–)	(+)
F2	(–)	(+)
F3	(–)	(Patient A)
F4	(–)	(+)
F5	(+)	(+)
F6	(+)	(–)
W1	(–)	(–)
W2	(–)	(–)
K1	(+)	(–)

Prepare Agarose Gels, TAE Buffer, and Stain (Fast Gel Protocol Only)

8. Prepare eight 1% TAE agarose gels with 8 wells each. Use 1x TAE (not 0.25x TAE) to make the gels. See Appendix B for gel casting instructions.

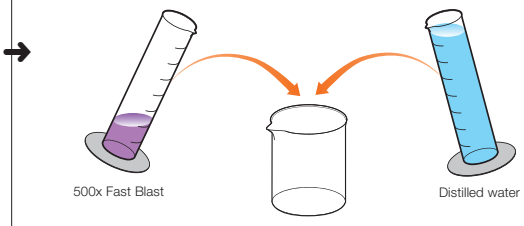
9. Prepare 2.2 L of 0.25x TAE electrophoresis buffer by adding 11 ml of 50x TAE to 2,200 ml of distilled water to use with the Fast DNA Gel Protocol.[2]

   Note: the correct volume of distilled water would be 2,189 ml, but the difference does not affect results.



10. If using, prepare 100x Fast Blast DNA stain for staining of agarose gels. See Appendix B for instructions.

    Note: Fast Blast DNA stain is not required if using UView 6x Loading Dye and Stain for visualization.



11. Print 8 copies of the Restaurant Staff and Patron Cards and 8 Restaurant Schematics. Provide these to each student group for Activity 3.

12. Download and familiarize yourself with the Student Guide for the virus you wish to focus on. **Do not tell students which transmission scenario they will be investigating** — they will apply their class results and what they know about the virus and possible modes of transmission to deduce the mode of transmission.

[2] This activity is designed to take advantage of Bio-Rad's Fast Gel Protocols using 0.25x TAE running buffer and higher voltages. See Appendix B for details and alternate electrophoresis options with preparation instructions.

Activity 1

Learning about Virus Biology, Pathophysiology, and Detection

This activity begins with an introduction to two patients who come into an emergency room with similar symptoms and conditions requiring diagnosis. It concludes with a review of a molecular diagnostic test that will be used to help in diagnosis.

It covers background information about viruses and has multiple curriculum connections to cell theory, biological molecules, the central dogma, body systems, polymerase chain reaction (PCR), and electrophoresis.

Classroom Preparation

- To save class time, assign the reading ahead of time and use the class time to go over the Focus Questions
- Prepare the slide deck and print the pages for Activity 1 from the Student Guide for students, or electronically distribute by PDF

Part 1: Patient Symptom Review

1. Review the data shown in Figure 1 and Table 1 of the Student Guide with students. Elicit their thoughts, questions, and prior knowledge about respiratory or gastrointestinal disease and the presentation of symptoms.

2. As a class, discuss student observations and record their ideas. "Do your observations justify ordering a diagnostic test? Why? What are the possible outcomes of not obtaining an accurate diagnosis?"

3. Have students review the reading and answer the Focus Questions.

Points to Emphasize

- The spread of a novel virus is of particular concern because the population will have no immunity against it, and the extent of disease and possible treatments may be unknown
- Different diseases and medical conditions can have similar symptoms, which can make diagnosis tricky
- Doctors rely on their experience, patient history, and diagnostic tests in making a diagnosis

Part 2: Learning about Viruses, Pathophysiology, and Detection

1. Review basic facts about viruses, emphasizing virus structure.

2. Ensure students understand (i) the difference between an enveloped and unenveloped virion, (ii) enveloped viruses require an intact lipid envelope to infect, and (iii) the genetic material within a virus can be DNA or RNA.

3. Compare and contrast coronaviruses and noroviruses.

4. Review the different types of molecular diagnostic tests, focusing on PCR-mediated tests.

5. As a class, review the Focus Questions.

6. Point out the career highlights that appear throughout this activity. Addressing a disease outbreak requires people from all sorts of different professions to work together and contribute their expertise.

Points to Emphasize

- The diversity of viruses makes it difficult to make general statements about them
- Only a small fraction of viruses cause disease in animals, plants, bacteria, or other organisms

Part 3: Patient Diagnosis

1. Review the RT-PCR diagnostic test for the scenario you will be using.

2. Reinforce that the test is looking for two different targets in a single tube. This means it uses two different sets of primers (multiplex assay).

3. Review the controls that will be used.

4. Review the basics of agarose gel electrophoresis and, as a class, answer the Focus Questions.

Points to Emphasize

- The virus in question in these activities is an RNA virus. This means the PCR-based diagnostic test requires a preliminary step of reverse transcription
- Each sample will produce one or two different bands: a band corresponding to human GAPDH (all samples will have this, and it shows the test worked) and a band corresponding to a target gene from the virus (only infected people will have this second band)
- Both PCR products (amplicons) will be possible in a single sample because the PCR test is a multiplex PCR test, meaning it has two different sets of PCR primers to allow amplification of two different targets in the same reaction

Instructor Background

Fun Facts about Viruses

The number of viruses that exist on our planet is staggering to think about. Roughly 10^{31} individual viral particles (virions) inhabit the oceans alone at any given time — 10 billion times the estimated number of stars in the observable universe (Dance 2021). Within this incredible number, there is also a lot of structural diversity — viruses exist in a wide range of sizes and shapes.
Points to emphasize with students:

- It is difficult to make general statements about viruses
- About 320,000 types of viruses are known to infect mammals; students will be focusing on only two in this activity
- All viruses do have two main things in common, but there are seemingly endless variations on these two main commonalities:
  - All have a nucleic acid genome encased in a protein-based shell (capsid)
  - All depend on a living host to reproduce
- Viruses tend to be characterized according to their shape, host cell, and/or genetic code (Pride 2020)
- Only a small fraction cause disease: in animal, plants, and bacteria

Molecular Diagnostic Tests

Diagnostic tests for viruses are based on laboratory techniques that can identify the presence of a viral infection in a patient. These tests can be divided into two main categories. The choice of test depends on the type of virus being detected, the stage of the infection, and the clinical context.

Detection of Viral Nucleic Acids (RNA or DNA)

These tests use techniques like PCR and reverse transcription PCR (RT-PCR) to look for the presence of viral DNA or RNA in a patient sample. Highly sensitive and specific, these tests can often detect viral infections even before symptoms appear. They do, however, require specialized equipment and often take longer to return results. Students may recognize these as the "PCR tests" used to test for COVID-19.

PCR and Reverse-Transcription PCR (RT-PCR)

PCR and RT-PCR are techniques used to amplify and detect specific DNA or RNA sequences. In this lab activity, students visualize the end-products of an RT-PCR test for a novel CoV or NoV. These types of tests are usually the fastest to be developed during an outbreak of a novel virus because they require only the creation of specific DNA primer sets. Developing primer sets is simple and quick once the genomic sequence of a virus is available.

- **PCR is used to amplify DNA sequences and so is used to detect DNA viruses.** It uses a DNA template, which is denatured by heat, and then two primers are annealed to the template. The primers then serve as starting points for a DNA polymerase enzyme to copy and amplify the DNA sequence between them. This process can be repeated multiple times, exponentially amplifying the original DNA sequence, allowing its detection
- **RT-PCR is used to amplify RNA sequences and so is used to detect RNA viruses.** RNA is first converted to DNA using the enzyme reverse transcriptase. The resulting DNA is then used as a template for PCR using DNA primers

Multiplex PCR

Be sure to point out to your students that the RT-PCR diagnostic test they are simulating is a multiplex RT-PCR. The test has two different sets of PCR primers to allow amplification of two different targets in the same reaction.

In their scenario, a single reaction is being used to detect the presence of more than one gene in a sample: the human GAPDH gene (all human samples should have this, showing the test worked) and a target gene from the virus (only infected people will have this second product).

Students who have previous experience with gel analysis of a PCR may confuse the presence of two bands in this lab activity with a restriction digestion. The Focus Questions in Activity 1 walk them through the results they should anticipate, but it is a good idea to repeat the concept of using two sets of primers in this fictitious test.

Detection of Viral Proteins or Patient Antibodies

These tests are used to diagnose past or present infections and can also help determine a patient's immune status. The most-used tests are enzyme-linked immunosorbent assays (ELISAs) and lateral flow immunoassays (LFIs). Students may recognize the latter as the rapid home tests used to test for COVID-19. Though often less sensitive and specific than PCR tests, these tests are convenient; they do not require specialized equipment, and they offer a shorter time to results.

Activity 2

Detecting Infections

In this activity, students 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. They then combine their results with results from their classmates in preparation for Activity 4.

Classroom Preparation

- Prepare student workstations (below)
- Print the pages for Activity 2 from the Student Guide for students
- Review the electrophoresis run conditions your students will use (Appendix B)

Student Workstation

Material	Quantity
Molecular weight ruler (MWR), 20 µl	1
GAPDH control (–), 10 µl	1
Virus- 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[3]	300 ml
100x Fast Blast DNA Stain (if using)	50 ml
1% TAE agarose gel with 8 wells[4]	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
Activity 2 printout	1

[3] Using 0.25x TAE buffer with a 1% agarose gel enables faster runs using higher voltage. See Appendix B for details.
[4] You may also use one gel with two rows of eight wells (2 x 8) for two student groups.

Part 1: Pre-Laboratory Questions

Students review their understanding of the diagnostic test and enter the purpose for each of their samples into Table 3.

Part 2: Patient Sample Analysis by Agarose Gel Electrophoresis

1. Review the available materials and procedure with students.
2. Introduce the expanded scenario: students will combine their results to get a complete picture of virus transmission in the restaurant.
3. Remind students to load 20 µl of MWR, 10 µl of control samples (–, +, and NTC), and 10 µl of each of their four participant DNA samples on their gel.
4. Ensure students record their sample numbers.
5. Instruct students which electrophoresis conditions to use (voltage and time settings; see Appendix B).
6. Have students conduct their investigations and visualize their DNA gels using Fast Blast DNA Stain or UView 6x Loading Dye and Stain (see Figure 1, below). Refer to Appendix B for visualization options and protocols.

Points to Emphasize

Accurate data recording is vital to any investigation. A common error is to incorrectly record what was loaded into each well of a gel.

Part 3: Data Analysis

This activity may be performed during the next class period as needed to accommodate schedules and gel staining method used. Have students sketch and interpret their gel results as directed in the Student Guide.

Part 4: Collecting Class Data

This activity may be performed during the next class period as needed to accommodate schedules and gel staining method used.

- Collect student data in a class data table or other shared space
- Have students summarize the class data in the tables in the Student Guide. Let them know which seat belonged to Patient A and have them indicate that spot as having tested positive

Agarose Gel Electrophoresis and Different Gel Stains

In real life, PCR-based tests are performed using instruments and other PCR-based technologies that provide readouts indicating infection status. For example, viruses are typically detected with quantitative real-time PCR (qPCR), which uses a different amplification chemistry and a type of thermal cycler that provides real-time, quantifiable readouts of results.

In this lab activity, however, students perform agarose gel electrophoresis to separate and visualize the PCR products.

A few notes regarding this procedure:

- **10-minute protocol** – DNA separation can be hastened on an agarose gel using modified conditions and alternate reagents; refer to Appendix B for options for running a gel in as little as 10 minutes
- **Smaller bands run together** – short separation times and distances will affect resolution of smaller bands; for this reason, the smallest of the MWR bands may run together and appear as a single, fuzzy, or wider band
- **Visualization/staining options** – this kit offers two visualization options; both are nontoxic options to ethidium bromide:
  - Fast Blast is used to stain DNA after separation in a gel (it stains the entire DNA-containing gel). It is economical but requires more time and requires use of loading dye, which is included with the kit
  - UView 6x DNA Loading Dye and Stain includes the loading dye and stains the DNA prior to separation. It offers instant visualization using a UV transilluminator or lamp

The gel appearance will vary slightly, depending on the method used (Figure 1).

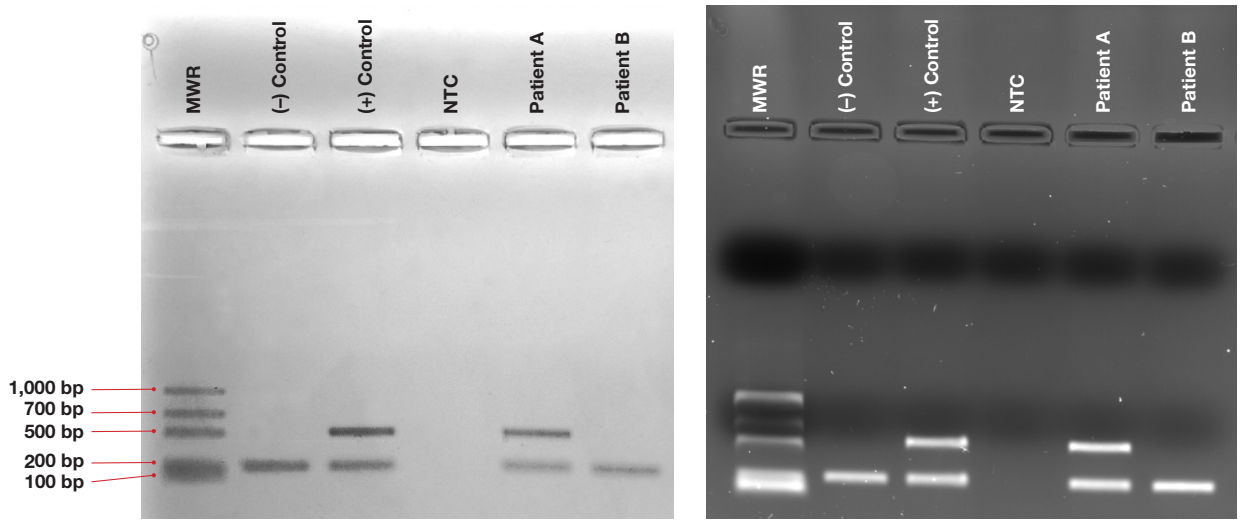


Figure 1. Comparison of agarose gels visualized using Fast Blast Stain (left) and UView 6x Loading Dye and Stain (right). Both gels were run using the 10-minute run protocol. Note the smallest bands in the MWR have not resolved completely. Note also that the dye in the UView 6x Loading Dye and Stain separates and creates its own dark bands which can mask bands in the MWR and samples if the separation time is insufficient.

Activity 3

Building a Transmission Model

In this activity, students learn about the chain of infection. They then analyze the classroom set of data from the restaurant, apply other patient and restaurant information, and propose an explanation for how the virus spread.

Classroom Preparation

- Print a restaurant layout and set of Restaurant Staff and Patron Cards for each workstation (see Appendix C)
- Ensure students have the class results from Activity 2

Part 1: Understanding the Chain of Infection

1. Review basic facts about the chain of transmission.

2. Ensure students understand how a mode of transmission may relate back to the structure of the virus of interest.

3. As a class, review the Focus Questions.

This review may also be done outside of class by the students in preparation for Part 2.

Part 2: Creating a Transmission Model

1. Have students apply their class results from Activity 2 to the restaurant layout.

2. Have students apply any additional information they may think is important from the restaurant staff and patron cards to the restaurant layout.

3. Who was Patient A? Invite students to explain why the Patient A sample was not included in their class analysis. (Patient A was tested and diagnosed at the hospital.)

4. Have the students propose a mode and route of transmission of the virus of interest in the restaurant. Students should consider all information provided, including the dimensions and features of the restaurant.

5. Direct students to support their claim with evidence from the restaurant and using other information they know about the type of virus.

Points to Emphasize

Differences in virus structure impact their modes of transmission and infection. Enveloped viruses must maintain an intact lipid envelope and envelope proteins to infect a host cell, which means the virion must remain hydrated. Enveloped viruses, therefore, are more likely to spread through respiratory droplets or aerosols or other bodily fluids like blood.

6. Invite students to build a chain of transmission using as many of the points in a chain as they can identify.

7. Ask students to examine the data and highlight any patterns or aspects of the data that support or conflict with their proposal.

8. Invite students to propose additional experiments epidemiologists might use to test the model for how transmission occurred.

Points to Emphasize

Though students may be looking for a primary mode of transmission, emphasize that in most cases, transmission can occur by multiple modes, for example fomite and food contamination, both aerosol and droplet transmission, droplet, and fomite, etc.

Instructor Background

About Coronavirus Transmission

This activity is based on a real-world case study of coronavirus transmission in a restaurant (Lu et al. 2020). Though coronaviruses are often transmitted through respiratory droplets or aerosols (or both), other modes of transmission are also possible.

About Norovirus Transmission

Noroviruses are highly contagious; <100 viral particles may be sufficient to infect a person. They are transmitted primarily through the fecal–oral route, either by consumption of contaminated food or water or by spreading directly from person to person. Shellfish and salad ingredients are the foods most often implicated in norovirus outbreaks, and many norovirus outbreaks have been traced to food handled by only one infected person.

In Quebec, Canada, in 2017, an outbreak of norovirus sickened more than 700 people. The culprit was imported frozen raspberries, so Canadian authorities issued a recall on raspberries and raspberry products from the region in question (Sherwood 2020).

Activity 4 (Optional)

Mitigating Risk

This activity correlates only to the coronavirus scenarios and is optional. It explores the considerations public health officials must make in determining whether and how to respond to the outbreak of a novel coronavirus.

Classroom Preparation

- This optional activity covers information about how communities might respond to an outbreak of a novel virus
- Prepare the slide deck and print the pages for Activity 4 from the Student Guide or distribute PDF electronically

Part 1: Understanding Response Strategies to Disease Outbreaks

1. Show students the data in Figure 12 and Table 1 of the Student Guide. Elicit their thoughts and questions about an epidemic curve.

2. Have students discuss the impacts and potential risks of allowing uncontrolled spread, especially of a novel virus, to answer the Focus Question.

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

1. Review basic facts the prevention strategies in Table 1 to elicit their thoughts and questions about the relative efficacy of each strategy and how they might be combined.

2. Answer the Focus Questions.

Ordering Information

Kits and Consumables

Catalog #	Description
17008261EDU	Virus Detection and Transmission Kit
17008251EDU	Virus Detection and Transmission Kit plus Small Fast Blast Electrophoresis Pack
17008241EDU	Virus Detection and Transmission Kit plus UView Electrophoresis Pack
1660450EDU	Small Fast Blast DNA Electrophoresis Reagents Pack, includes 25 g agarose powder, 100 ml 500x Fast Blast DNA Stain, 100 ml 50x TAE electrophoresis buffer
1660462EDU	Small UView DNA Electrophoresis Reagents Pack, includes 25 g agarose powder, 1 ml UView 6x Loading Dye and Stain, 100 ml 50x TAE electrophoresis buffer
1613116EDU	Certified Molecular Biology Agarose, 5 g
1613100EDU	Certified Molecular Biology Agarose, 25 g
1660742EDU	TAE Electrophoresis Buffer, 50x, 100 ml
1665111EDU	UView 6x Loading Dye and Stain, 200 µl
1665112EDU	UView 6x Loading Dye and Stain, 1 ml
2239480EDU	1.5 ml EZ Micro Test Tubes, clear, 500
1613057EDU	1% TAE Mini ReadyAgarose Precast Gel, 7.1 x 10 cm, 2 x 8-well
1613015EDU	1% TAE Mini ReadyAgarose Precast Gel, 7.1 x 10 cm, 8-well

Equipment

Catalog #	Description
1660551EDU	Classroom Adjustable-Volume Digital Micropipet, 2–20 µl
1660512EDU	Fixed-Volume Micropipet, 10 µl
1660506EDU	Professional Adjustable-Volume Digital Micropipet, 2–20 µl
1660507EDU	Professional Adjustable-Volume Digital Micropipet, 20–200 µl
1660531EDU	UView Mini Transilluminator
1645050EDU	PowerPac Basic Power Supply
1664000EDU	Mini-Sub Cell GT Cell Tank and Lid
1660481EDU	Green Racks, pkg of 5

Fast Gel Protocol and Electrophoresis Preparation Instructions

Fast Gel Protocol

There are multiple ways to hasten visualization of DNA bands on an agarose gel using modified conditions and alternate reagents.

Options, as well as the required materials and protocols, are provided below.

1. Cast 1% agarose gels with 1x TAE buffer.

   Note: you may also use precast agarose gels (for example, 1613015EDU or 1613057EDU).

2. Prepare 0.25x TAE electrophoresis buffer.

3. Load samples, run gel using conditions in the table below, and visualize DNA using one of the stain options below.

Electrophoresis Buffer and Voltage	Electrophoresis Time
0.25x TAE at 300 V*	10 min
0.25x TAE at 200 V	20 min
1x TAE at 100 V	30 min

* Requires power supply capable of voltages over 200 V, such as the PowerPac Basic Power Supply (1645050EDU).

Fast Blast DNA Stain: Prepare DNA samples before electrophoresis with 5x Orange G Loading Dye. After electrophoresis, stain gels with Fast Blast DNA Stain and visualize the next day.

UView 6x Loading Dye and Stain: Prepare DNA samples for electrophoresis using 6x UView Loading Dye and Stain. After gel electrophoresis, visualize instantly with a UV transilluminator or a handheld UV lamp in the dark.

Preparing Agarose Gels

For each class of eight workstations, prepare either (i) eight 7 x 7 cm gels with one 8-well comb, or (ii) four 7 x 10 cm gels with two 8-well combs. Use the volumes and quantities of reagents shown in the table below.

Volumes and Quantities of Reagents for Agarose Gels.

Number of Gels	1	4	8	16
1% TAE Agarose Gel (7 x 7 cm) — serves one workstation				
Purified water, ml	39	156	312	624
50x TAE, ml	0.8	3.2	6.4	12.8
Agarose, g	0.4	1.6	3.2	6.4
Total, volume of molten agarose, ml	40	160	320	640
1% TAE Agarose Gel (7 x 10 cm) — serves two workstations				
Purified water, ml	49	196	392	784
50x TAE, ml	1.0	4.0	8.0	16.0
Agarose, g	0.5	2.0	4.0	8.0
Total, volume of molten agarose, ml	50	200	400	800

Prepare Molten Agarose

1. Add the appropriate amount of agarose powder and then the liquids to a suitable container; fill to less than 75% of the container volume. Swirl to mix.

   Note: If using an Erlenmeyer flask, place a small, inverted 25 ml flask over the opening to minimize evaporation. If using a bottle, loosen the cap to allow air and steam to escape.

2. Place the agarose solution into the microwave. Microwave for 3 min. Continue to boil in 30 sec increments until the solution boils and all agarose has dissolved.

   Caution: Always wear heat-protective gloves, goggles, and a lab coat while preparing agarose gels. Hot molten agarose can cause severe burns.

3. Let the agarose cool to 60°C before pouring the gels.

Cast Agarose Gels

There are a variety of ways to cast agarose gels. This section outlines the tape method. Consult the instruction manual for your horizontal electrophoresis system for alternate methods.

1. Firmly seal the ends of a gel tray with standard laboratory or masking tape (not regular sticky tape).

2. Place the comb into the appropriate slot in the gel tray. If pouring a double-well gel, place a comb at one end of the tray and another in the middle.

3. Once the molten agarose has cooled to at least 60°C, pour enough agarose to cover ¾ of the way up the gel comb teeth or to a depth of 0.5–0.75 cm.

4. Allow the gel to solidify at room temperature for 10–20 min; it will be opaque when ready to use.

5. Carefully lift the combs straight up to remove them. Carefully remove the tape.

6. Store gels in a sealable plastic bag with a few ml of buffer at room temperature for up to 1 day, or in the refrigerator for up to 1 week.

Preparing TAE Buffer

Though 1x TAE buffer is usually used both for gel casting and as running buffer, the electrophoresis time can be reduced by running the gels with 0.25x TAE buffer at 200 or 300 V. When using this faster protocol, gels should still be cast using 1x TAE buffer.

You will need 300 ml buffer for each gel. Combine distilled water with the volume of 50x TAE buffer indicated and mix well.

Volumes and Quantities of Reagents for Agarose Gels.

Number of Electrophoresis Chambers	1	4	8	16
0.25x TAE Buffer				
Purified water, ml	274	1,094	2,189	4,378
50x TAE, ml	1.4	5.6	11	22
Total, volume of 0.25x TAE buffer, ml	275	1,100	2,200	4,400

Note: If you are reusing 0.25x TAE buffer between classes, ensure the buffer is at or below room temperature before reuse. If the buffer starts out warm, it may become hot enough to melt the agarose gel during a high-voltage run.

Visualizing DNA

UView 6x Loading Dye and Stain

No additional preparation is needed when using UView 6x Loading Dye and Stain.

- Replace Orange G Loading Dye with UView 6x Loading Dye and Stain when preparing DNA samples
- Directly after electrophoresis, carefully place gels on a UV transilluminator, lower UV shield, and turn on UV light to visualize
- Cell phones can be used to capture gel images: Turn off the flash and place transilluminator in a dark room or area

Fast Blast DNA Stain

1. Prepare 100x Fast Blast DNA Stain according to volumes in the table below.

100x Fast Blast DNA Stain Preparation.

Number of 7 x 10 cm Gels to Stain	1	4	8	16
500x Fast Blast DNA Stain, ml	10	40	100	200
Distilled water, ml	40	160	400	800
Total volume of 100x Fast Blast DNA Stain, ml	50	200	500	1,000

2. Add ~50 ml of 100x stain per gel in a gel staining tray and gently rock for 2–3 min.

3. After 2 min, pour off stain and retain for future use.[5] Staining longer will increase background.

4. Rinse gel with tap water for 30 sec to 1 min or until all surface stain is removed.

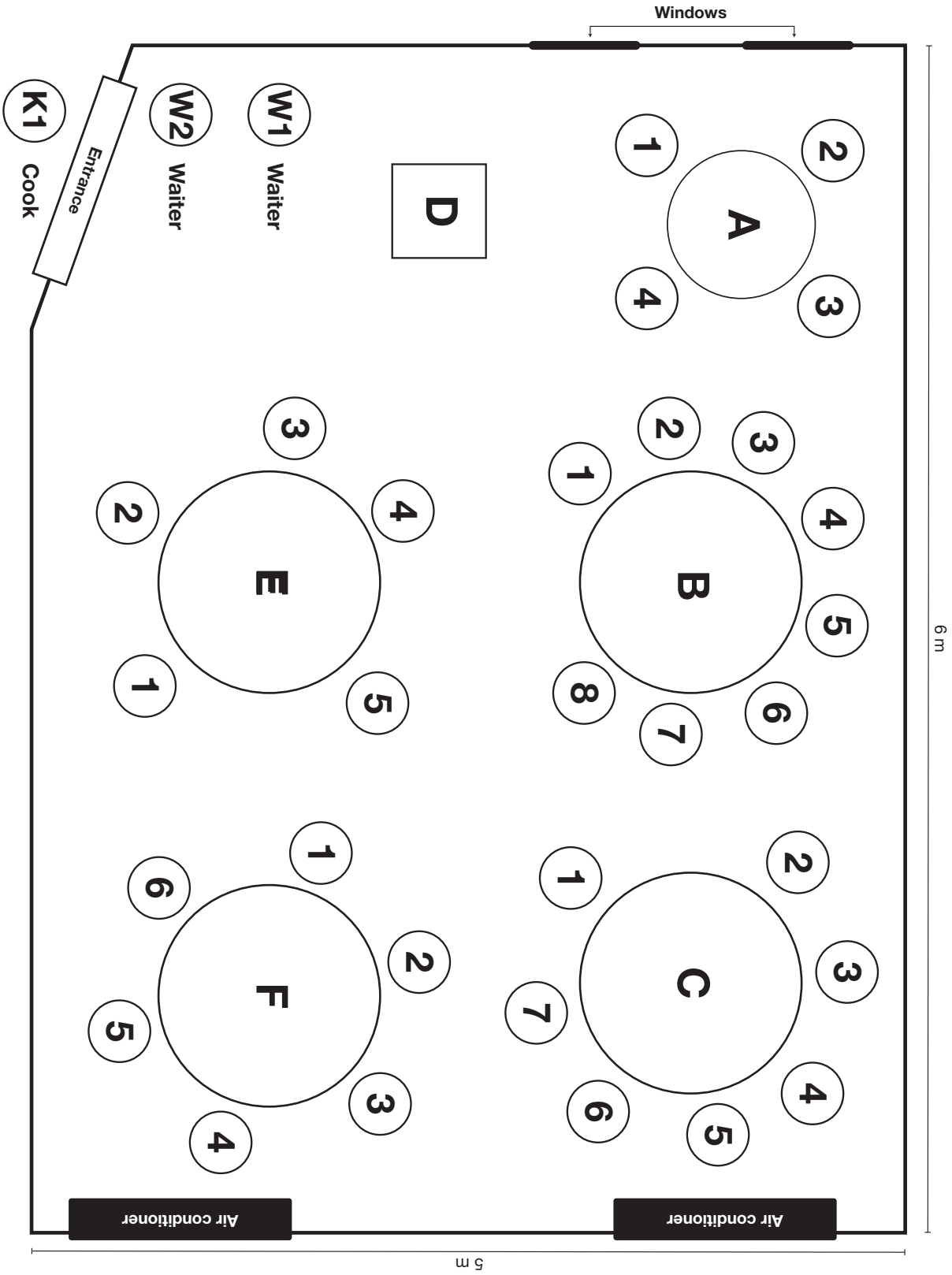
5. Cover the gel with a large volume of tap water and gently rock to destain. DNA will be visible as dark blue bands against a lighter blue background after a few hours, with contrast gradually increasing overnight. Destain overnight for best results.

   Note: Using 100x Fast Blast DNA stain prevents the small DNA fragments from diffusing in the gel during an overnight destaining.

[5] 100x Fast Blast DNA Stain can be reused at least six times.

Restaurant Layout

Several independent air conditioners were running in the room, which created isolated and recirculating streams of air. The windows to the outdoors were closed. Note no one was seated at Table D.



Restaurant Staff and Patron Cards

Patient Background

Name: Tav (male) 26

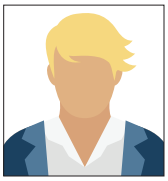
Seat: A1

Back story: Roommate of A2. Lives in a neighboring town. Met friends for lunch.

Restroom: N/A

Food at restaurant: Onion rings, mushroom sandwich, soft drink

Patient Background



Name: Aidan (male) 27

Seat: A2

Back story: Roomate with A1. Lives in a neighboring town. Met friends for lunch.

Restroom: 12:48 pm

Food at restaurant: Clam chowder, onion rings, iced tea

Patient Background

Name: Ezri (female) 28

Seat: A3

Back story: Friend of A1 and A2. Lives nearby. Met friends for lunch.

Restroom: N/A

Food at restaurant: Raw oysters, mushroom sandwich, water

Patient Background

Name: Madison (female) 26

Seat: A4

Back story: Friend of A1–A3. Lives nearby. Met friends for lunch.

Restroom: N/A

Food at restaurant: Fish tacos, french fries, soft drink

Patient Background

Name: Connor (male) 68

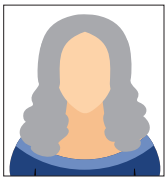
Seat: B1

Back story: Father of B3. Lives nearby. Met friends for lunch.

Restroom: 12:31 pm

Food at restaurant: Clam chowder, garlic pasta, hot tea

Patient Background



Name: Arabella (female) 65

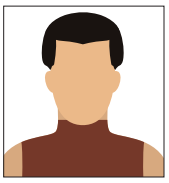
Seat: B2

Back story: Mother of B3. Lives nearby. Met friends for lunch.

Restroom: N/A

Food at restaurant: Shared raw oysters with B3, pasta, iced tea

Patient Background



Name: Zach (male) 23

Seat: B3

Back story: In town visiting family. Lives in another region. Arrived in town on June 2.

Restroom: N/A

Food at restaurant: Shared raw oysters with B2, fish tacos, onion rings, iced tea

Patient Background

Name: Maciah (male) 39

Seat: B4

Back story: Brother of B3, married to B5. Lives in the region, but not nearby.

Restroom: N/A

Food at restaurant: Shrimp cocktail, cheeseburger, soft drink

Patient Background

Name: Riley (female) 38

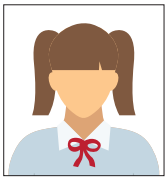
Seat: B5

Back story: Spouse of B4.
Lives in the region, but not nearby.

Restroom: N/A

Food at restaurant: Vegetable soup, white fish fillet, iced tea

Patient Background



Name: Kaley (female) 10

Seat: B6

Back story: Daughter of B4 and B5.
Lives in the region with her parents, but not nearby.

Restroom: 12:45 pm

Food at restaurant: Split shrimp cocktail, garlic pasta, lemonade

Patient Background

Name: Camielle (female) 66

Seat: B7

Back story: Aunt of B3 and sister of B1.
Lives nearby.

Restroom: N/A

Food at restaurant: Garden salad, clam chowder, hot tea

Patient Background

Name: Jake (male) 66

Seat: B8

Back story: Uncle of B3 and
brother-in-law of B1. Lives nearby.

Restroom: N/A

Food at restaurant: Fried oyster sandwich, soft drink

Patient Background

Name: DaeLynn (female) 69

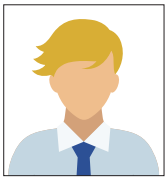
Seat: C1

Back story: Mother of of C4 and C5.
Lives with C3 in a neighboring region. Visiting family.

Restroom: 12:15 pm

Food at restaurant: Shrimp cocktail, mushroom sandwich, water

Patient Background



Name: Matteo (male) 14

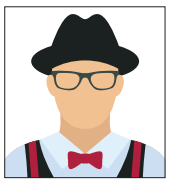
Seat: C2

Back story: Child of C5. Lives with C5.

Restroom: 1:08 pm

Food at restaurant: Fish tacos, french fries, lemonade

Patient Background



Name: Jackson (male) 70

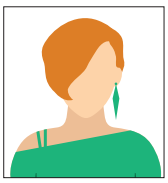
Seat: C3

Back story: Grandfather. Lives with C1
in a neighboring region. Visiting family.

Restroom: N/A

Food at restaurant: Fried oyster sandwich, seasoned french fries, water

Patient Background



Name: Devlin (female) 42

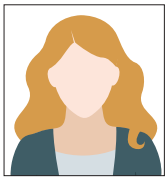
Seat: C4

Back story: Sister of C5. Lives nearby.

Restroom: N/A

Food at restaurant: Calamari, garden salad, mushroom sandwich, iced tea

Patient Background



Name: Hollis (female) 45

Seat: C5

Back story: Mother of C2 and C6. Lives nearby.

Restroom: 12:20 pm

Food at restaurant: Calamari, roast chicken, hot tea

Patient Background

Name: Conway (male) 17

Seat: C6

Back story: Child of C5. Lives with C5.

Restroom: N/A

Food at restaurant: Cheeseburger, seasoned fries, lemonade

Patient Background

Name: Delaney (female) 47

Seat: C7

Back story: Friend of C5. Lives in a neighboring town.

Restroom: 12:36 pm

Food at restaurant: Garden salad, tuna sandwich, iced tea

Patient Background

Name: Tim (male) 54

Seat: E1

Back story: Works nearby with everyone at table E. Lives in a different region. Eating before a work function.

Restroom: N/A

Food at restaurant: Vegetable soup, roast chicken, iced tea

Patient Background

Name: Nick (male) 36

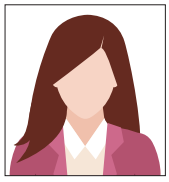
Seat: E2

Back story: Works nearby with everyone at table E. Lives in neighboring town. Eating before a work function.

Restroom: 12:53 pm

Food at restaurant: Clam chowder, french fries, hot tea

Patient Background



Name: Kiki (female) 42

Seat: E3

Back story: Works nearby with everyone at table E. Lives nearby. Eating before a work function.

Restroom: N/A

Food at restaurant: Shrimp cocktail, hot pastrami sandwich

Patient Background

Name: Savannah (female) 43

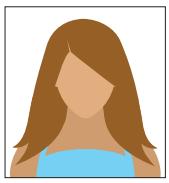
Seat: E4

Back story: Works nearby with everyone at table E. Lives in a different region. Eating before a work function.

Restroom: 12:09 pm

Food at restaurant: Raw oysters, mushroom sandwich, soft drink

Patient Background



Name: Neenah (female) 38

Seat: E5

Back story: Works nearby with everyone at table E. Lives in a neighboring town. Eating before a work function.

Restroom: N/A

Food at restaurant: Vegetable soup, hot pastrami sandwich, water

Patient Background

Name: Ricardo (male) 17

Seat: F1

Back story: Attends a school near the restaurant. Friends with F2–F6. Lives nearby. Celebrating the birthday of F3. Ate a cupcake from F2.

Restroom: N/A

Food at restaurant: Tuna sandwich, soft drink

Patient Background

Name: Alex (male) 17

Seat: F2

Back story: Attends a school near the restaurant. Friends with all at table F. Lives nearby. Celebrating the birthday of F3. Brought homemade cupcakes from home to share. Ate one himself.

Restroom: 12:26 pm

Food at restaurant: Cheeseburger, split an order of onion rings, soft drink

Patient Background

Name: Anna (female) 18

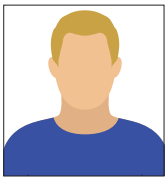
Seat: F3

Back story: Attends a school near the restaurant. Friends with all at table F. Lives nearby. Celebrating her birthday with friends. Ate a cupcake from F2.

Restroom: N/A

Food at restaurant: Fish tacos, split an order of onion rings, lemonade

Patient Background



Name: Stefan (male) 17

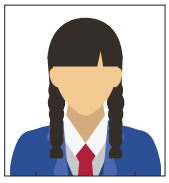
Seat: F4

Back story: Celebrating the birthday of F3. Ate a cupcake from F2.

Restroom: 1:03 pm

Food at restaurant: Vegetable soup, split onion rings, water

Patient Background



Name: Naija (female) 17

Seat: F5

Back story: Attends a school near the restaurant. Friends with all at table F. Lives nearby. Celebrating the birthday of F3. Ate a cupcake from F2.

Restroom: N/A

Food at restaurant: Garden salad, split an order of fries, lemonade

Patient Background

Name: Brooklyn (female) 18

Seat: F6

Back story: Attends a school near the restaurant. Friends with all at table F. Lives nearby. Celebrating the birthday of F3. Ate a cupcake from F2.

Restroom: N/A

Food at restaurant: Vegetable soup, split an order of fries, water

Patient Background

Name: Elijah (male) 23

Seat: W1

Back story: Arrived at 11 am.
Worked lunch shift, primarily serving tables E and F. Began feeling sick midway through the shift and went home at 1:15 pm.

Restroom: N/A

Food at restaurant: N/A

Patient Background

Name: Dannie (female) 24

Seat: W2

Back story: Arrived at at 10:45 am for an 11:30 am open. Prepped dining room. Worked all day, primarily serving tables A, B, and C.

Restroom: 1:30 pm, 4:26 pm

Food at restaurant: N/A

Patient Background

Name: Anthony (male) 33

Seat: K1

Back story: Arrived at work at 8:30 am. Received food shipment. Prepped ingredients. Cooked all meals.

Restroom: 9:30 am, 11 am, 4:56 pm

Food at restaurant: N/A

Resources

Organization	Link/Reference	Type of Resource	Description
Bio-Rad	bio-rad.com/vdtkit	Supplementary documentation	Instructor Guide, Student Guide, bulletins
	bio-rad.com/classroomresources	Supplementary documentation	YouTube playlists, supplementary resources
	Answer Guide (included with kit)	Supplementary documentation	Answers to Focus Questions
Centers for Disease Control and Prevention (CDC)	CDC NERD Academy Student Quick Learn: How Does Disease Spread? (youtu.be/1QLgXzyXOH0)	Video	Describes how infection prevention specialists use information on the type of infectious agent, how it is spread, and common ways it exits and enters its hosts to create a chain of infection model
	Lesson 1: Introduction to Epidemiology, Section 10: Chain of infection (cdc.gov/csels/dsepd/ss1978/lesson1/section10.html)	Website	Describes the chain of infection
	COVID-19 Outbreak Associated with Air Conditioning in Restaurant, Guangzhou, China, 2020 wwwnc.cdc.gov/eid/article/26/7/20-0764-f1	Website	Describes the transmission case and restaurant layout upon which these kit activities are based
Professor Dave Explains	Viruses: Molecular Hijackers (youtu.be/wUgEhfo_qxU)	Video	Describes viruses, what they are and how they cause disease
	Introduction to Virology and Viral Classification (youtu.be/IADC0C_WeH0)	Video	Describes viruses, what they are, how they were discovered, variations in nature
	Routes of Viral Transmission (youtu.be/S5egQXgBZ6c)	Video	Describes various modes and routes of transmission
Washington Post	Stevens, H (2020) Why outbreaks like coronavirus spread exponentially, and how to “flatten the curve” (www.washingtonpost.com/graphics/2020/world/corona-simulator/)	Website with simulator	Offers a deeper description of the epidemic curve
El Pais	Salas J and Jafra M (2020) An analysis of three Covid-19 outbreaks: how they happened and how they can be avoided (elpais.com/especiales/coronavirus-covid-19/an-analysis-of-three-covid-19-outbreaks)	Web simulation	Describes several settings for outbreaks studied by health authorities with conclusions that offer insights into mitigation strategies

Bibliography

Cassedy A, et al. (2021). Virus detection: A review of the current and emerging molecular and immunological methods, Front Mol Biosci 8: 637559.

Hardstaff JL, et al. (2018). Foodborne and food-handler norovirus outbreaks: A systematic review, Foodborne Pathog Dis 15: 589–597.

Jayaweera M, et al. (2020). Transmission of COVID-19 virus by droplets and aerosols: A critical review on the unresolved dichotomy, Environ Res 188: 109819.

Li Y, et al. (2021). Probable airborne transmission of SARS-CoV-2 in a poorly ventilated restaurant, Build Env 196, 107788.

Louten J (2021). Virus transmission and epidemiology, Essential Human Virology 2016: 71, 92.

References

Dance A (2021). The incredible diversity of viruses, Nature 595: 22–25.

Pride D (2020). Viruses can help us as well as harm us, Sci Amer 323, 46–53.

Lu J, et al. (2020). COVID-19 outbreak associated with air conditioning in restaurant, Guangzhou, China, 2020, Emerg Infect Dis 26, 1628-1631.

Sherwood D (2020). How a Chilean raspberry scam dodged food safety controls from China to Canada, Reuters 6 October 2020.

BIO-RAD Explorer

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Website *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *00 800 00 24 67 23* ***Belgium*** *00 800 00 24 67 23* ***Brazil*** *4003 0399*
Canada *1 905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *00 800 00 24 67 23* ***Denmark*** *00 800 00 24 67 23* ***Finland*** *00 800 00 24 67 23*
France *00 800 00 24 67 23* ***Germany*** *00 800 00 24 67 23* ***Hong Kong*** *852 2789 3300* ***Hungary*** *00 800 00 24 67 23* ***India*** *91 124 4029300* ***Israel*** *0 3 9636050*
Italy *00 800 00 24 67 23* ***Japan*** *81 3 6361 7000* ***Korea*** *82 080 007 7373* ***Luxembourg*** *00 800 00 24 67 23* ***Mexico*** *52 555 488 7670*
The Netherlands *00 800 00 24 67 23* ***New Zealand*** *64 9 415 2280* ***Norway*** *00 800 00 24 67 23* ***Poland*** *00 800 00 24 67 23* ***Portugal*** *00 800 00 24 67 23*
Russian Federation *00 800 00 24 67 23* ***Singapore*** *65 6415 3188* ***South Africa*** *00 800 00 24 67 23* ***Spain*** *00 800 00 24 67 23* ***Sweden*** *00 800 00 24 67 23*
Switzerland *00 800 00 24 67 23* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *66 2 651 8311* ***United Arab Emirates*** *36 1 459 6150* ***United Kingdom*** *00 800 00 24 67 23*

10000164965 Ver A US/EG *23-0461 0723 Sig 0123*