EDVOTEK®

THE **BIOTECHNOLOGY**
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Edvo-Kit #
209

Edvo-Kit #209

Going Un-Viral: Virus Quantification Using Plaque Assays

Experiment Objective:

In this simulation experiment, students will use a viral plaque assay to determine the viral load of two patients undergoing antiviral treatment. They will then share classroom data to evaluate the treatment's effect.

See page 3 for storage instructions.

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Safety Data Sheets can be found on our website: **www.edvotek.com/safety-data-sheets**

Experiment Components

REAGENTS	Storage	Check (✓)
• *E.coli* BactoBeads™	4° C with desiccant	❑
• Bacteriophage T4 ViroBeads™	4° C with desiccant	❑
• ReadyPour™ Luria Broth Agar	Room Temp.	❑
• COLORTOP™ Agar	Room Temp.	❑
• 1X Phosphate Buffered Saline (PBS)	Room Temp.	❑
• Luria Broth	Room Temp.	❑

SUPPLIES

- 50 mL conical tube ❑
- 15 mL screw-cap conical tubes ❑
- 1.5 mL snap-top microcentrifuge tubes ❑
- Sterile loops ❑
- Petri plates (large) ❑
- Wrapped 10 mL pipet (sterile) ❑

Experiment #209 is designed for 10 lab groups.

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

Requirements *(not included with this kit)*

- 37° C incubation oven
- Water bath(s)
- Automatic micropipettes and tips
- Pipet pump
- White light box (optional)
- Aluminum foil

This lab simulates a viral load test for HIV on human HeLa cells using the benign and classroom optimized substitutes of Bacteriophage T4 ViroBeads™ and a non-pathogenic strain of *E. coli* BactoBeads™.

Background Information

WHAT IS A VIRUS?

In the late 1800s, scientists were working to characterize how microbes could cause disease. While some diseases like cholera, tuberculosis, and anthrax could be explained by the presence of specific bacteria, other diseases, like rabies, could not. In these cases, the diseases were considered to be caused by dangerous "biological chemicals" that were named viruses, from the Latin word for poison. An important breakthrough occurred when Dutch scientist Martinus Beijerinck proposed that the virus that caused tobacco mosaic disease was (1) a living agent that could replicate - but only inside tobacco plant cells - and (2) was much smaller than bacteria.

Today, scientists know that viruses are very simple infectious particles and can even observed these particles - known as virions - using powerful electron microscopes (Figure 1). Virions are comprised of a DNA or RNA genome surround by a protective protein coat and, in some cases, a lipid cover. When not inside a host cell, they range in size from five to three hundred nanometers and vary tremendously in shape (Figure 2). Because viruses carry genetic material, reproduce, and evolve but rely entirely on a host organism for basic biological functions - like transcription and metabolism - they are often described as organisms on the border between life and chemistry. In fact, depending on who you ask viruses are or are not alive.

Viruses replicate by using the machinery and metabolites (enzymes, ribosomes, nucleotides, ATP, etc.) of a host cell. This pathogenic process can be divided into five key stages (Figure 3).

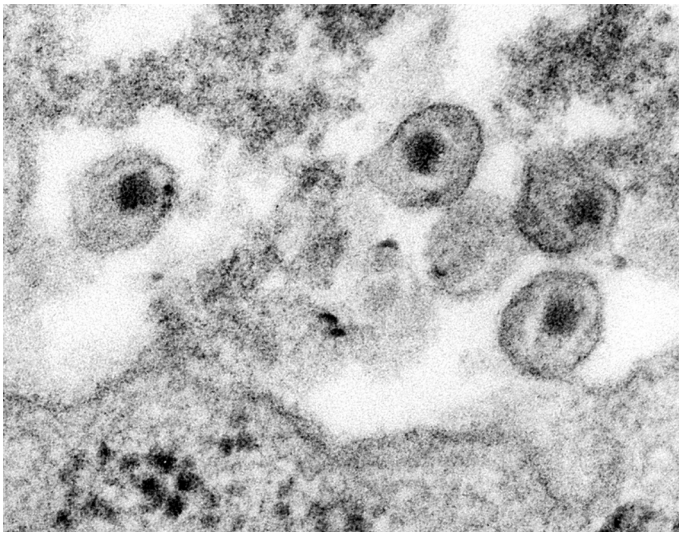


Figure 1: An electron microscope image of the Human Immunodeficiency Virus.

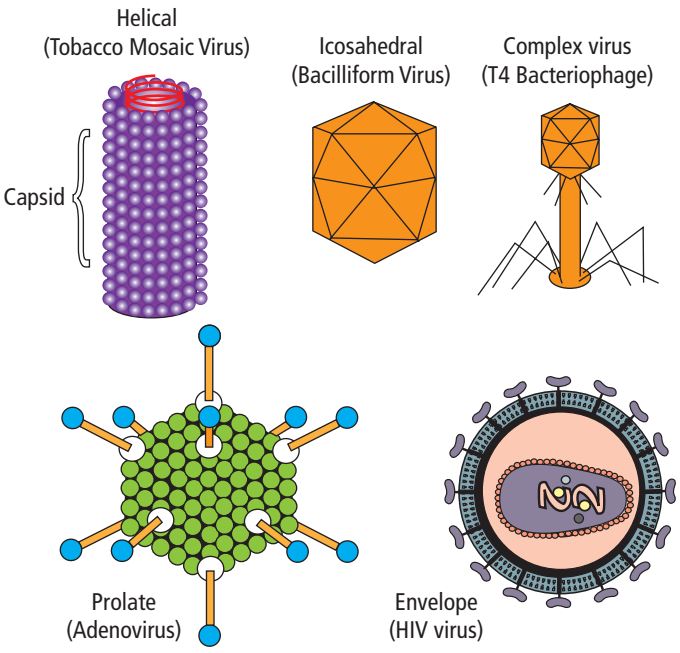


Figure 2: Structure of Viruses

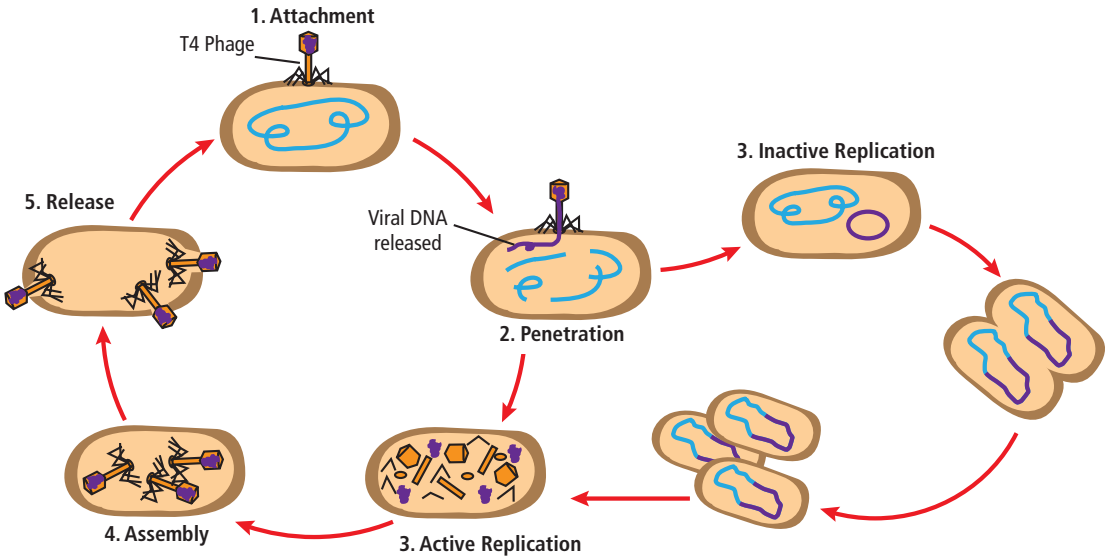


Figure 3 - Viral Life Cycle

(1) **Attachment**
The virus binds to a host cell, which causes the viral and cellular membranes to fuse. Because this binding is highly specific, viruses often target one or two host organisms and specific cells within these organisms.

(2) **Penetration**
The virus either enters the cell and releases its genetic material or it injects its genetic material into the cell while its exterior shell remains outside.

(3) **Replication**
The virus either uses the host cell machinery to produce viral RNA, proteins and additional genome copies (active replication) or it integrates its genome with the host genome to become a provirus (inactive replication). A provirus virus is copied and passed to daughter cells each time a cell divides.

(4) **Assembly**
Newly synthesized viral parts self assemble into new virions.

(5) **Release**
The new virions are released by the host cell either through a process of lysis (which kills the cell) or by budding.

Viral replication is highly successful. Consequently, viruses are both ancient and prolific. Evolutionary studies estimate that viruses appeared roughly 3.4 billion years ago - right around the time that cells evolved. Today, they are by far the most common biological unit on earth and likely outnumber all other types of life put together. One diversity study that looked at the number of viruses on a square meter of exposed surface estimated that approximately 800 million new viruses arrived each day! A huge number of viruses also live inside of us. In fact, there may be 100 times more viruses than cells in most individuals! The majority of these viruses are benign and some may even have coevolved to be beneficial. However, others are harmful to their human hosts. These virulent viruses represent a major area of medical research. Virology is a specialization of microbiology that explores viruses and the diseases that they cause.

VIRUSES AND HUMAN HEALTH

When viruses use human cells to reproduce the results can range from unnoticeable to uncomfortable to extremely dangerous. For example, around 200 virus species have been linked to the common cold. These viruses attack cells in the upper respiratory system sometimes within 15 minutes of entering the body but are eventually eradicated by a healthy immune system. (Most of the symptoms of a cold - coughing, sneezing, fever, inflammation, and fatigue - are due to the immune system's response.) At the other end of the spectrum are viruses belonging to the genus *Ebolavirus, Rabies,* and *Variola* which cause the often fatal diseases of Ebola, Rabies, and Small Pox.

Some of the most threatening human viruses are those that can switch between an active and symptomatic phase and an inactive or latent infection. In the latter state, the virus is "hidden" within the host's cells as a provirus. This makes it difficult for the immune system to detect and attack the virus and also makes it difficult to treat the infection with drugs or other therapies without also injuring the host. Examples of this type of virus are the Varicella Zoster Virus (which causes Chicken Pox and Shingles), Herpes Simplex Virus (which causes Herpes), and the Human Immunodeficiency Virus (which causes AIDS).

For all viral infections, the first line of treatment is prevention. This starts by limiting the spread of viruses. Washing hands, monitoring foods, using safe sex practices, and even putting on bug spray are all examples of this type of defense. Another defense is vaccination. Our immune systems are incredibly effective at dealing with dangerous viruses if they can recognize and attack them early. A viral vaccine contains a weakened form of the virus, a part of the virus, or a synthetic imitation of the virus that stimulates the bodies' immune response without inducing the disease. Following immunization with a vaccine, an individual's immune system can quickly recognize and destroy the real virus if it ever enters the body.

Antiviral drugs are a post-infection treatment option. However, because viruses use the host cell to replicate, it is often difficult to find effective drugs that interfere with the virus without harming the host. In addition, viruses can adapt to a drug and become resistant. To combat this, doctors will often prescribe a cocktail containing multiple drugs when treating an infection. Currently, around 100 antiviral drugs exist. Most of these drugs disrupt the viral replication cycle by limiting a virus' ability to attach to cells, incorporate its genetic material into the host, or synthesize new genetic material. Although some antiviral drugs have been developed for the flu, most are used to treat chronic infections like Hepatitis, Herpes, and AIDS. In these cases, the goal is for the drug to keep the virus inactive and undetectable in the body rather than to completely cure an individual.

VIRAL QUANTIFICATION USING A PLAQUE ASSAY

Doctors prescribing antivirals for chronic infections need to continuously monitor the severity of the infection. They do this by determining the concentration of viral particles in a given sample. This value is often referred to as the viral load, the viral burden, or the viral titer. For example, the viral load of an individual with AIDS who is undergoing HIV antiviral therapy will be determined before the treatment, 2 to 8 weeks after the start of treatment, and then every 3-6 months as long as the treatment continues. Such monitoring helps the doctor evaluate the success of the therapy and quickly detect anti-viral resistance. Knowing their viral load also helps patients better manage their condition and make decisions about activities that could potentially spread an active virus.

There are many methods for measuring viral load. One highly accurate method is to measure the number of specific viral DNA or RNA sequences in a sample using either quantitative PCR or reverse transcriptase PCR. This method requires access to both advanced equipment and highly trained technicians. Another popular tool is Enzyme-linked Immunosorbent Assays (ELISAs), which use antibody-antigen binding to detect specific anti-viral proteins in a patient's sample. ELISAs are quick and sensitive but are only indirect measures of viral load. Finally, a plaque assay can be used to determine viral load based on the number of cells infected by active viruses in a patient's sample.

During a plaque assay, the potentially infected sample is serially diluted and inoculated into a host cell culture. This is followed by an incubation period that allows any virions present to attach to these new cells (Figure 4a). Next, a layer of agar is poured over the potentially infected cells. Because the host cell culture is a single layer of cells

and because the agar blocks the virus from moving vertically the only way for a virus to spread is by infecting a neighboring cell (Figure 4b). Consequently, during the next incubation period, a circular zone of infection will form around any cell that was infected (Figure 4c). This circular zone is known as a plaque and can, in fact, be visualized by the naked eye – particularly when additional dyes that emphasize the difference between uninfected, living cells and infected, dead cells are used.

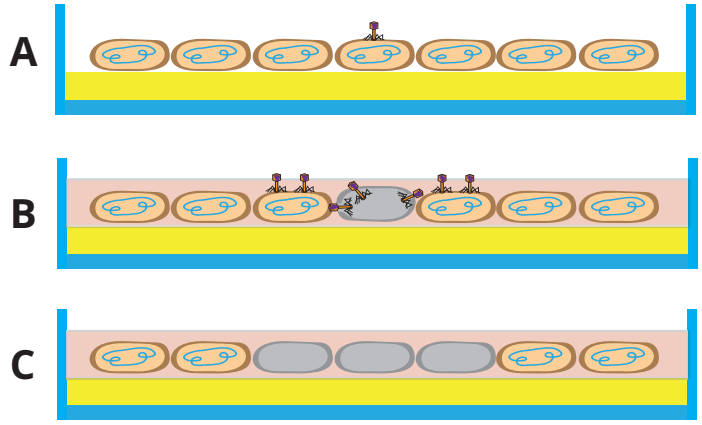


Figure 4 - Viral Plaque Assays

Following a plaque assay, the viral load is calculated as a measure of plaque forming unit (PFU) per milliliter. To account for variation and errors in counting, only plates containing between 10 and 100 plaques are used to calculated PFU measurements. In addition, multiple plates are prepared per sample because the viral concentration in a patient can vary tremendously over time (Figure 5). For example, someone who has recently been infected with HIV can have over 40 million viral copies per mL of blood while someone on treatment with an undetectable viral load can have less than 50 viral copies per mL. To account for this range scientists will prepare a serial dilution of the initial sample, create a plate for each dilution, and then incorporate the dilution strength into their PFU calculations.

In this lab, your class will be testing two individuals who are taking a new combination of anti-HIV drugs. Blood samples were taken before and during the treatment. Each group will prepare four plaque assay plates in order to determine the viral load for one patient at one time point. As a class, you will then share your results and evaluate the success of the treatment for both patients.

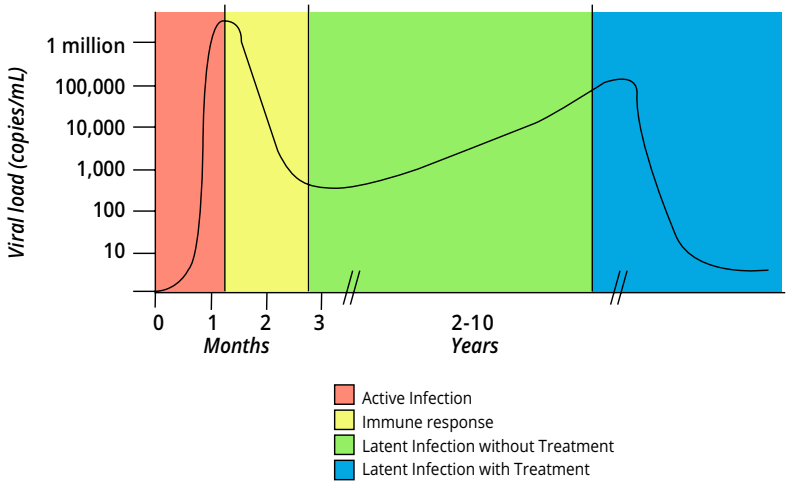


Figure 5 - Viral Titration Changes Over Time

Experiment Overview

EXPERIMENT OBJECTIVE:

In this simulation experiment, students will use a viral plaque assay to determine the viral load of two patients undergoing antiviral treatment. They will then share classroom data to evaluate the treatment's effect.

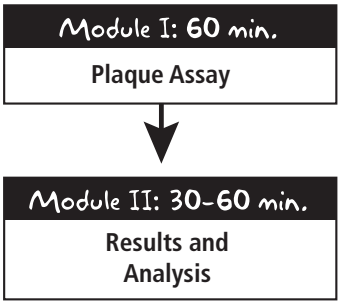
LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

Wear gloves and safety goggles

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.



Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

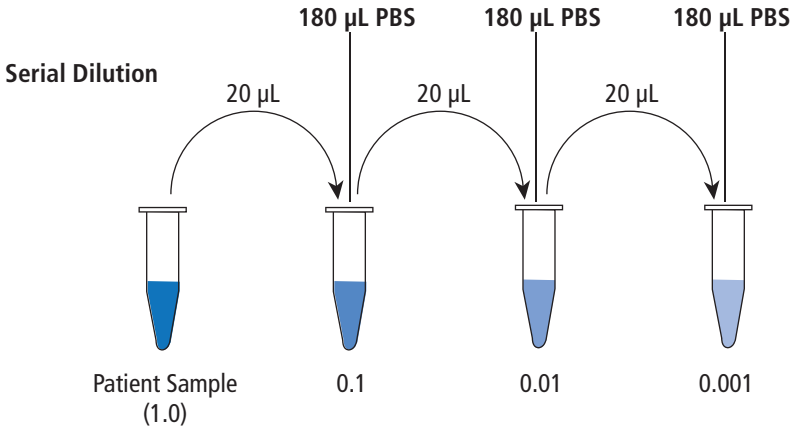
After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Module I: Plaque Assay

In this module you will be preparing a serial dilution of a patient's sample. You will then perform a plaque assay on each dilution using a double agar overlay method. A mixture of virus, bacteria, and COLORTOP™ soft agar is prepared and then poured over a hard agar base. Finally, you will incubate the plates for 24 hours to allow the virus to spread and infected cells to rupture.

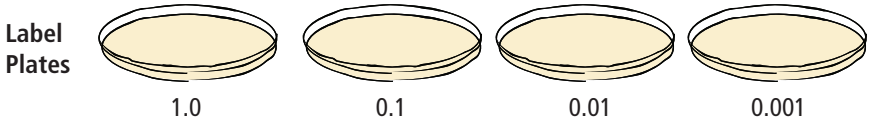
PREPARE A SERIAL DILUTION SERIES OF THE PATIENT SAMPLE




Wear gloves and safety goggles

1. **LABEL** three microcentrifuge tubes as "0.1", "0.01", and "0.001". (This is the dilution factor.) Also **LABEL** each tube with your group ID.
2. **ADD** 180 µL of 1X PBS to each of these three tubes.
3. **COLLECT** a patient sample from your teacher. This is your 1.0 dilution.
4. **ADD** 20 µL of the patient sample to the 0.1 tube. **MIX** by pipetting up and down or by flicking the tube.
5. **ADD** 20 µL of the 0.1 sample to the 0.01 tube. **MIX** by pipetting up and down or by flicking the tube.
6. **ADD** 20 µL of the 0.01 sample to the 0.001 tube. **MIX** by pipetting up and down or by flicking the tube.

LABEL AGAR PLATES



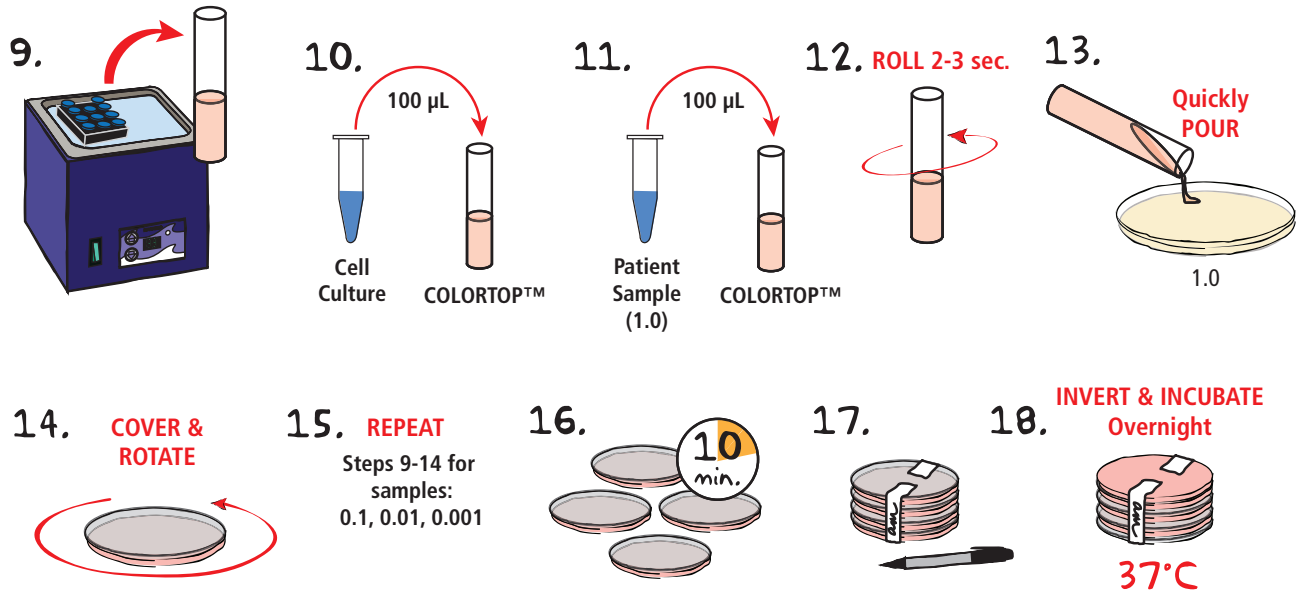
7. **CHECK** that all plates were at 37° C. The assay will only work when the base layer is warm.
8. **LABEL** four plates as "1", "0.1", "0.01" and "0.001". Also **LABEL** each plate with your group ID, the patient ID, and the time point. Keep labels small and towards the edge in order to easily view plaques later on.

NOTE: Keep labeled plates warm by either returning them to the incubator and removing them one at a time OR by stacking them and loosely wrapping the stack with foil.

Module I: Plaque Assay, continued

PERFORM THE PLAQUE ASSAY

The instructions below are for the original patient sample (dilution factor of 1) but should be repeated for the three serial dilutions. Because the color top agar cools very quickly, prepare only one plate at a time. Also perform steps 9-14 as rapidly as possible.



9. **COLLECT** a tube of COLORTOP™ Agar from the water bath or incubator.
10. **ADD** 100 µL of the cell culture to the tube.
11. **ADD** 100 µL of the patient sample (dilution factor of 1) to the tube.
12. **ROLL** the agar tube between your palms for 2-3 seconds to mix the cell culture and the virus with the agar.
13. **POUR** the mixture onto the pre-warmed agar plate labeled "1.0".
14. **COVER** the plate and **LIFT** the plate from the bench. Holding the plate in your hands, gently **ROCK** and **ROTATE** the plate in a circular motion. This disperses the COLORTOP™ agar evenly across the surface of the LB agar.
15. **REPEAT** steps 9 through 14 for samples 0.1, 0.01, and 0.001.
16. **ALLOW** the soft agar overlay of all four plates to harden for at least 10 minutes.
17. **STACK** the hardened plates on top of one another, tape them together, and label the tape with your group ID.
18. **PLACE** the plates in an inverted position (agar side on top) and **INCUBATE** overnight at 37° C.

WARNING!
The cell culture and patient samples must be added, mixed, and poured quickly to prevent hardening of the COLORTOP™ Agar.

OPTIONAL STOPPING POINT:
Following incubation, plates can be placed in a zip lock bag and saved for 1-2 weeks at 4° C.

Module II: Results and Analysis

In this module you will observe the four plates you prepared in Module I, count the number of plaques on each plate, and use this to determine the viral load of the original patient sample. Next, you will use class data to plot viral load changes over time in two patients and recommend any treatment changes.

CALCULATE YOUR SAMPLE'S VIRAL LOAD

1. **OBSERVE** your plates. The plates should be cloudy with cell growth. Plaques will appear as small clear spots.
2. When possible, **COUNT** the number of plaques on each plate. Because of the wide range of dilutions some plates may have no plaques and others may have too many plaques to count. **RECORD** the numbers in the table below.
3. **CALCULATE** the number plaque forming units per milliliter (PFU/mL).
   a. Select a plate that has more than 10 but fewer than 100 plaques. (If there are two plates that fit within this range calculate the viral load for both and average the results.)
   b. Multiply the dilution factor of the plate by the volume of virus added to the plate in Module I step II (100 µL = 0.1 mL).
   c. Divide the number of plaques on the plate by the number calculated in the previous step.

PATIENT_____, TIME_____

Plate	Observations	# of Plaques
1.0		
0.1		
0.01		
0.001		

Viral Load*_____

$$*\textit{Viral Load} = \frac{\textit{PFU}}{\textit{mL}} = \frac{\textit{\# of Plaques}}{\textit{(Dilution Factor x Volume of Diluted Virus Added)}}$$

DETERMINE A TREATMENT PLAN (OPTIONAL)

4. **SHARE** your data as a class to determine both patients' viral load at all 5 time points.

Time	Patient 1 Viral Load (PFU/mL)	Patient 2 Viral Load (PFU/mL)
Before Treatment (T0)		
1 months after Treatment (T1)		
3 months after Treatment (T2)		
6 months after Treatment (T3)		
9 months after Treatment (T4)		

5. **GRAPH** the results with time after treatment on the X-axis and PFU/mL on the Y-axis.
6. **ANSWER** the following: Does the virus appear to be suppressed in both patients? Would you advice continued treatment or a revised treatment approach for patient 1? What would you advice for patient 2?

Study Questions

1. Choose a virus that has been in the news recently either because of its human health or economic toll. Research and find out the viruses shape and size. Next draw a simple sketch of the virion and label its genetic material, protein coat, and lipid cover (if present).

2. The debate continues around whether or not viruses are living organisms. Fill out the table below with at least 4 bullet points. (Choose a side or make an argument for both sides.)

Living Characteristics of Viruses	Nonliving Characteristics of Viruses

3. Each year the flu costs the US economy billions in sick days and lost productivity and endangers many of our most vulnerable citizens. Outline three treatment approaches that could help reduce the number of flu cases or the severity of infections.

4. Why is it important to create several different dilutions of a patient's sample before preforming a plaque assay?

Instructor's Guide

OVERVIEW OF INSTRUCTOR'S PRELAB PREPARATION

This section outlines the recommended prelab preparations and approximate time requirements to complete each prelab activity. This experiment is designed for 10 groups.

Preparation For:	What to do:	When:	Time Required:
Module I: Plaque Assay	Pour LB Base Plates	2-7 Days before Module I	45 min.
	Prepare PBS	Before Module I	5 min.
	Prepare Host Cell Culture	Day before Module I	10 min. plus overnight incubation
	Prepare Patient Samples	Day before Module I	30 min.
	Equilibrate water bath at 60° C and incubator at 37° C. Warm LB plates.	Day of Module I	10 min.
	Prepare COLORTOP™ Agar and control plates.	Day of Module I	50 min.
Module II: Results and Analysis	Procure access to graphing software (OPTIONAL)	Anytime before Module II	Varies

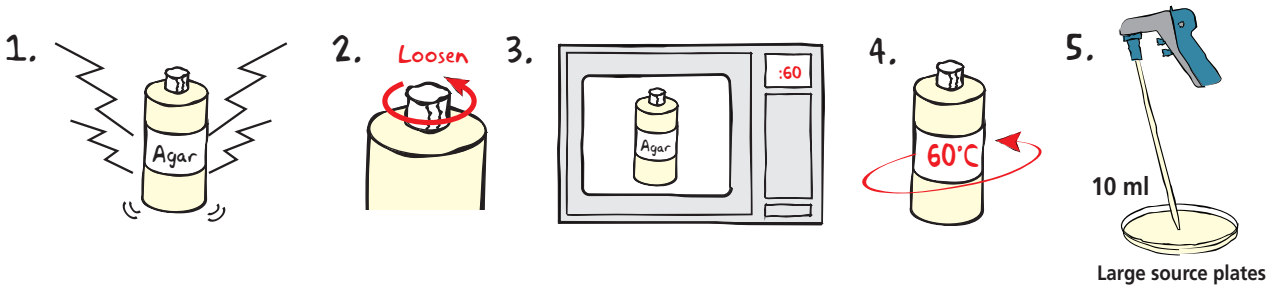
Red = Prepare immediately before module. Yellow = Prepare shortly before module. Green = Flexible / prepare up to a week before the module.

IMPORTANT:
Make sure both you and your class are familiar with and practice basic aseptic technique throughout the prelab and Module I as both the LB Agar and COLORTOP™ Agar can become contaminated. Contamination does not affect plaque formation but can make counting more difficult. Good practices include:

- Sterilizing lab benches with 10% bleach or 70% ethanol.
- Capping tubes and covering plates quickly and whenever possible.
- Wearing disposable lab gloves.
- Using sterile pipette tips particularly when preparing the LB plates and aliquoting the COLORTOP™ Agar.

Pre-Lab Preparations

POUR LB AGAR BASE PLATES (2-7 DAYS BEFORE MODULE I)



Large source plates

1. **BREAK** solid ReadyPour™ LB Agar into small chunks by vigorously squeezing and shaking the plastic bottle.
2. **LOOSEN**, but **DO NOT REMOVE**, the cap on the ReadyPour™ Agar bottle. This allows the steam to vent during heating. **CAUTION:** Failure to loosen the cap prior to heating may cause the bottle to break or explode.
3. **MICROWAVE** the ReadyPour™ Agar on high for 60 seconds to melt the agar. Carefully **REMOVE** the bottle from the microwave and **MIX** by swirling the bottle. Continue to **HEAT** the solution in 30-second intervals until the agar is completely dissolved (the amber-colored solution should be clear and free of small particles).
4. **COOL** the ReadyPour™ Agar to 60° C with careful swirling to promote even dissipation of heat.
5. **POUR** 10 mL of the cooled ReadyPour™ Agar into each of the forty-two large petri dishes using a 10 mL pipet and pipet pump.
6. **COVER** and **WAIT** at least twenty minutes for the LB-agar plates to solidify. For optimal results, leave plates at room temperature overnight.
7. **STORE** plates at room temperature for no more than two days. Plates should be inverted and placed in a sealable plastic bag to ensure that they do not dry out.

Wear Hot Gloves and Goggles during all steps involving heating.

NOTE for Step 3:
Use extra care and make sure the agar does not boil out of the bottle. Pay close attention and stop the heating if it starts to bubble up.

NOTE: If plates are prepared more than two days before use, they should be stored inverted in a plastic bag in the refrigerator (4° C). Remove the plates from the refrigerator and warm in a 37° C incubator before use.

Quick Reference: Pouring LB Agar Plates

- Use a sterile 10 mL pipet with a pipet pump to transfer the designated volume of medium to each petri plate. Pipet carefully to avoid forming bubbles.
- Rock the petri plate back and forth to obtain full coverage.
- If the molten medium contains bubbles, they can be removed by passing a flame across the surface of the medium.
- Cover the petri plate and allow the medium to solidify.

Pre-Lab Preparations, continued

Prepare PBS (Before Module I)

1. **LABEL** ten snap-top microcentrifuge tubes with "1X PBS".
2. **ALIQUOT** 1 mL 1X PBS into each tube.
3. **SAVE** remaining PBS for patient samples and optional control plates.

Prepare Host Cell Culture (Day Before Module I)

1. Using a sterile loop, **ADD** two BactoBeads™ to 10 mL of LB in a 50 mL conical tube.
2. **INCUBATE** for 16-20 hours in a 37° C incubator.
3. **ALIQUOT** 500 µL of the cell culture into ten snap top tubes.
4. **SAVE** remaining cell culture for optional control plates.

Prepare Patient Samples (Day Before Module I/Day of Module I)

The instructions below create four concentrations that simulate one patient responding well to an antiviral treatment and one patient showing signs of antiviral resistance. However, you can create your own scenario and dilutions.

1. **UNCAP** the ViroBeads™ vial.
2. Using a sterile pipet tip, **ADD** 1.25 mL of sterile 1X PBS to the vial. **MIX** by pipetting up and down several times. This is your viral stock solution.
3. In a snap top tube, **COMBINE** 50 µL of stock solution and 450 µL of sterile PBS to create a 1:10 dilution. **MIX** well.
4. In a snap top tube, **COMBINE** 10 µL of stock solution and 490 µL of sterile PBS to create a 1:50 dilution. **MIX** well.
5. In a 15 mL tube, **COMBINE** 10 µL of stock solution and 5 mL of sterile PBS to prepare a 1:500 dilution. **MIX** well.
6. Use Table 1 to **LABEL** ten 1.5 mL snap top tubes and **ALIQUOT** 130 µL of the appropriate dilution to each tube.
7. If samples are prepared the day before, **KEEP** at 4° C.

Sample	Treatment Timeline	Dilution
P1, T0	Before treatment	Stock
P1, T1	1 month after treatment	1:50
P1, T2	3 months after treatment	1:500
P1, T3	6 months after treatment	1:500
P1, T4	9 months after treatment	1:500
P2, T0	Before treatment	Stock
P2, T1	1 month after treatment	1:50
P2, T2	3 months after treatment	1:10
P2, T3	6 months after treatment	1:10
P2, T4	9 months after treatment	1:10

Table I: Dilutions of ten patient samples.

Warm LB Agar Base Plates (Day of Experiment)

1. **INCUBATE** previously prepared agar base plates at 37° C for 15-30 minutes before performing the experiment. The plates MUST be warm for the experiment to work properly.
2. Each student group should **COLLECT** four plates right before Module I step 7.

Pre-Lab Preparations, continued

Prepare COLORTOP™ Agar (Day of Module I)

1. **PREPARE** water bath(s). For this experiment you will need to **IMMERSE** forty-two 15 mL screw-top conical tubes in 60° C water. One way to accomplish this is to cover the top of the water bath(s) with foil and poke holes for each 15 mL tubes. Using this method 42 tubes require ~375 cm^2 of exposed water bath (two Edvotek® 1.8L, one Edvotek® 10 L).
2. **LOOSEN**, but DO NOT REMOVE the cap on the COLORTOP™ agar bottle. This allows the steam to vent during heating.
3. **MICROWAVE** the COLORTOP™ Agar on high for 30 seconds to melt the agar. Carefully **REMOVE** the bottle from the microwave and **MIX** by swirling the bottle. Continue to heat the solution in 15 second intervals until the agar is completely dissolved (the red-colored solution should be clear and free of small particles.)
4. Once melted, **DISPENSE** 5 mL of agar into each 15 mL screw-top conical tube (4 tubes per group x 10 groups + 2 optional controls = 42 tubes.)
5. **PLACE** all 15 mL tubes in 60° C water bath(s) to keep the agar from solidifying. The agar MUST be kept warm at all times.
6. Student groups should **COLLECT** tubes one at a time during Module I steps 9 - 14.

FOR MODULE I
Each group should receive:

1 Snap top tube of 1 mL of 1x PBS
1 Snap top tube of 500 µL Cell Culture
1 Snap top tube of 130 µL Patient Sample
3 Snap top tubes
4 LB agar base plates, INCUBATED AT 37° C
4 Conical tubes containing 5 mL COLORTOP™ Agar, INCUBATED AT 60° C
1 Small square of foil (to insulate base plates)

Prepare Control Plates (Day of Module I) (OPTIONAL)

Additional ReadyPour, COLORTOP™ Agar, and Petri Plates have been provided for you to prepare two negative controls of uninfected cell cultures. Such controls are used in a clinical setting to check cellular viability and help in plaque identification. They are also an opportunity for you to become familiar with the plaque assay technique before the class.

1. **COLLECT** a tube of COLORTOP™ Agar from the water bath and a LB agar plate from the incubator.
2. **ADD** 100 µL of the cell culture to the tube.
3. **ADD** 100 µL of 1X PBS to the tube.
4. **ROLL** the agar tube between your palms for 2-3 seconds to **MIX** the bacteria and the 1X PBS with the agar.
5. **POUR** the mixture onto the pre-warmed agar plate.
6. **COVER** the plate.
7. **LIFT** the plate from the bench. Holding the plate in your hands gently **ROCK** and rotate the plate in a circular motion. This disperses the COLORTOP™ agar evenly across the surface of the agar.
8. **REPEAT** steps 1 – 7 for a second control.
9. **ALLOW** the soft agar overlay of both plates to harden for at least 10 minutes.
10. **PLACE** the plates in an inverted position (agar side on top) and incubate overnight at 37° C along with the student plates from Module I.

PRE-LAB PREPARATIONS FOR MODULE II

To graph class data, students will need access to graphing software.

Example Student Data

VIRAL LOAD CALCULATION

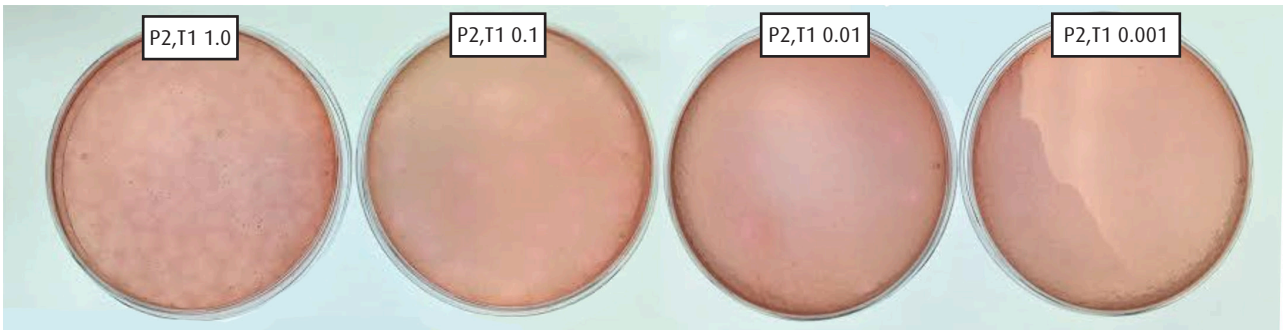


Plate	Observations	# of Plaques
1.0	Many plaques through out the plate, most have merged.	Too many to count
0.1	Many plaques through out the plate, a few have merged.	39
0.01	A few isolated plaques.	6
0.001	No plaques. Continuous layer of cells easily identifiable.	0

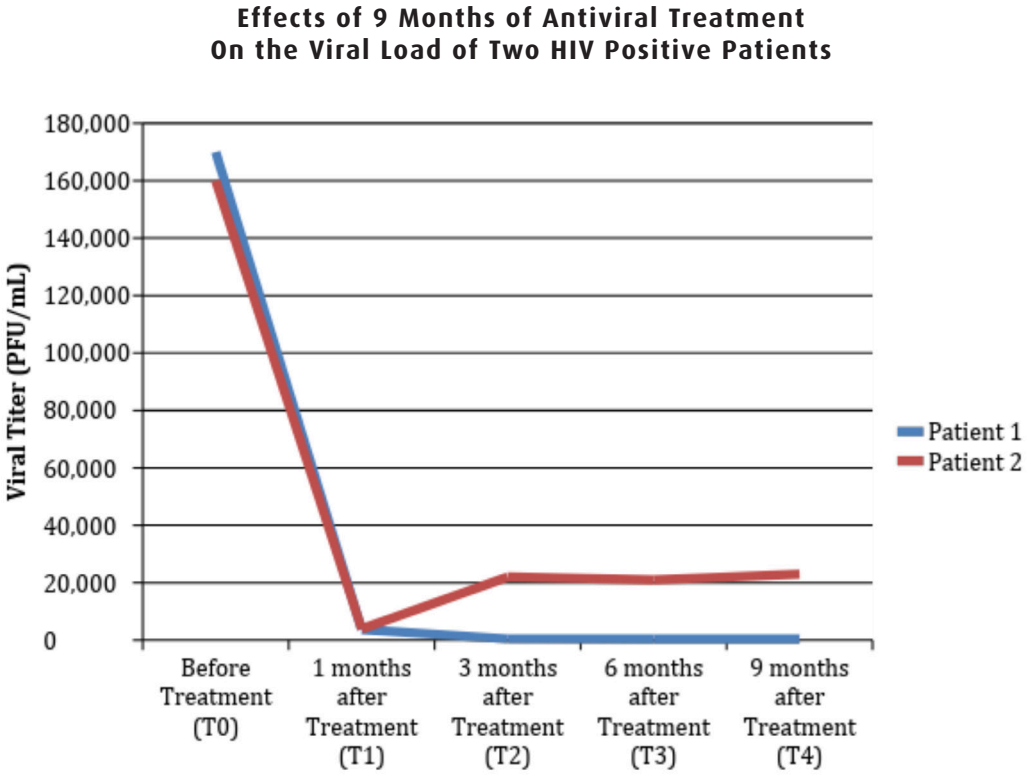
In this example, only the 0.1 dilution plate had a count between 10 and 100. The viral load on this plate was 39 plaques. This is divided by the dilution factor of the original patient sample (0.1) and the amount added to the plate (100 µL) in mL (0.1 ml).

Viral Load = 39 PFU/(0.1*0.1 mL) = 3,900 PFU/ml.

DETERMINE A TREATMENT PLAN (OPTIONAL)

Time	Patient 1 Viral Load (PFU/mL)	Patient 2 Viral Load (PFU/mL)
Before Treatment (T0)	170,000	160,000
1 months after Treatment (T1)	3,700	3,900
3 months after Treatment (T2)	480	22,000
6 months after Treatment (T3)	440	21,000
9 months after Treatment (T4)	450	23,000

Example Student Data, continued



For patient 1 the viral load dropped and has since stayed at a low level for many months. This indicates a stable and successful treatment that is keeping the virus suppressed and has stopped the progression of the HIV disease. In this case, doctors would recommend continuing antiviral treatment and monitoring every 3 months. After 2 years the frequency of this monitoring may be decreased to every 6 months.

For patient 2 the viral load initially dropped but now appears to be increasing. This indicates that the current treatment regime is unable to control the HIV infection. Reasons for the viral rebound may be poor adherence to the treatment regime, low absorption of the medications, unexpected drug-drug interactions, or the evolution of drug-resistant viruses. In this case, a doctor would test to determine the underlying cause by seeing if/what drug-resistant viruses are present and by discussing any treatment changes or challenges with the patient. Luckily a number of HIV antivirals have been developed and a second, alternative treatment may be successful.

Please refer to the kit insert for the Answers to Study Questions

Appendix A

EDVOTEK® Troubleshooting Guide

PROBLEM:	CAUSE:	ANSWER:
Cell lawn does not form.	Excessive heat deactivated the virus or killed the cells.	The COLORTOP™ agar should be cooled to 60°C before adding the virus/cell mixture.
Plaques do not form.		
Plaques concentrated in one area of the plate.	Virus/cell solution not thoroughly mixed into the COLORTOP™ agar before the overlay was poured.	Be sure to spread the COLORTOP™ agar across the entire surface of the agar base plate by moving the plate in a circular direction. The COLORTOP™ agar remains liquid for a longer period of time when the base plate is heated, making it easer to spread the agar.
	COLORTOP™ agar was not spread evenly across the surface of the plate.	
	Base plate was not properly warmed before pouring the COLORTOP™ agar.	
Plaques are all different shapes and sizes.	Natural Variation	Although the majority of plaques should be round, they can take differnent shapes. Count all distinct regions of clearing regardless of the shape and size.
Lumpy, bubbly and/or uneven overlay	COLORTOP™ agar was below 60°C when it was poured on the plate.	COLORTOP™ agar should be warm enough to avoid hardening while adding to the plate to avoid lumps and bubbles in agar.
	Base plate was not properly warmed before pouring the COLORTOP™ agar.	COLORTOP™ agar may solidify too quickly if it comes into contact with a cold base plate. This makes it difficult to evenly spread the agar.