Edvo-Kit #124

DNA Screening for Smallpox

Experiment Objective:
The objective of this experiment is to develop an understanding of Smallpox and the causative agent of the disease. Students will analyze simulated PCR products to confirm or rule out the presence of the Smallpox virus.

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](http://www.edvotek.com/safety-data-sheets)
Experiment Components

READY-TO-LOAD™ SAMPLES FOR ELECTROPHORESIS

Store QuickStrip™ samples in the refrigerator immediately upon receipt. All other components can be stored at room temperature.

Components (in QuickStrip™ format)  Check (√)
A  Standard DNA Marker
B  Simulated Smallpox positive control marker
C  Negative control
D  Cowpox control DNA sample
E  Sample from Patient #1
F  Sample from Patient #2

REAGENTS & SUPPLIES

- UltraSpec-Agarose™
- Electrophoresis Buffer (50x)
- 10x Gel Loading Solution
- FlashBlue™ DNA Stain
- InstaStain® Blue cards
- 1 ml pipet
- Microtipped Transfer Pipets

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipets with tips
- Balance
- Microwave, hot plate or burner
- Pipet pump
- 250 ml flasks or beakers
- Hot gloves
- Safety goggles and disposable laboratory gloves
- Small plastic trays or large weigh boats (for gel destaining)
- DNA visualization system (white light)
- Distilled or deionized water

Experiment #124 is designed for 8 gels if stained with FlashBlue™ or InstaStain® Blue (both included) or 16 gels if stained with SYBR® Safe or InstaStain® Ethidium Bromide (not included).

Store QuickStrip™ samples in the refrigerator immediately upon receipt. All other components can be stored at room temperature.

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.
Background Information

Smallpox is a serious, highly contagious disease characterized by severe skin eruptions. These eruptions leave pitted scars, known as “pocks”, which is the basis of the word “pox”. Throughout history, it is estimated that smallpox has killed over 100 million people and left another 200 million blind and/or permanently scarred. The first documented cases of smallpox occurred over 3000 years ago in China, Egypt, and India. The Egyptian pharaoh Ramses V died of smallpox in 1157 B.C. The plague of Antonine, approximately AD 180, was a smallpox epidemic that killed over 3 million people and coincided with the first stages of the decline of the Roman Empire. From 1512-1515, 3.5 million Aztecs in Mexico died from smallpox after being exposed to the disease by a single Spaniard accompanying the conquistador Hernando Cortez. In Europe in the late 18th century, smallpox killed over 400,000 people annually with an additional third of the survivors struck blind. Even as recently as the 1960s, the disease has infected over 10 million and killed over 2 million people worldwide annually.

Due to the high fatality and high communicability, many attempts have been made to use smallpox as a weapon. During the Middle Ages in Europe and Asia, bodies of smallpox victims were often catapulted over the walls of a city under siege in the attempts to start an epidemic in the attacking army. In the 16th century, it is believed that the Spanish conquistador Francisco Pizarro gave smallpox-infected clothing to native South Americans. In 1763, during the French-Indian War, Lord Jeffrey Amherst, the British commander-in-chief for America, distributed smallpox-laden blankets (from a local smallpox hospital) to enemy Indian tribes, killing one-third of their population. During the American Civil War, Dr. Luke Blackburn attempted to infect clothing with smallpox that he then sold to Union troops, although the success of this tactic was unclear, as infectious disease was already rampant among the soldiers. During World War II, the United States and Japan worked on weaponizing smallpox. In the late 1980s, the Soviet Union embarked on an ambitious project to place smallpox in intercontinental ballistic missiles, and reportedly produced hundreds of tons of the virus.

VARIOLA VIRUS

The virus that causes smallpox, known as variola, belongs to a family of viruses known as Poxviridae, recognizable in the microscope by their brick-shaped structures. Smallpox is divided into two subtypes: variola major, with a mortality rate of 20-40%, and variola minor, a less serious form of the disease, with a mortality rate of 1-2%. Chickenpox is a very common childhood virus resembling smallpox. It is caused by the Varicella-Zoster virus and is more superficial and has less serious skin eruptions. Monkeypox is native to Central and West Africa and is usually transmitted to humans by contact with primate blood or after suffering a bite. Monkeypox is moderately transmissible from human to human. Cowpox is not as serious and usually results from direct contact with the infected udder or teats of cattle and horses.

Smallpox and the other viruses carry their genetic material as DNA and replicate in the cytoplasm of infected cells. Variola virus has a genome size of 190 kilobases and consists of 200 genes. The virion is composed of over 100 proteins and carries its own RNA polymerase which transcribes the viral DNA. Virus assembly occurs in the cytoplasm, followed by host cell lysis with release of thousands of virus particles, resulting in extremely rapid multiplication of the virus during the incubation period.

Variola virions are quite stable in saliva aerosol droplets and usually enter the patient through the respiratory tract. Following a 10-15 day incubation period, symptoms begin to appear. Headache, severe back pain, high fever, prostration, and delirium are early symptoms. Following infection of internal organs, the virus re-enters the bloodstream and infects the skin (approximately 3 days after the initial onset of symptoms). This skin infection results
in the characteristic smallpox rash which starts as small red dots that proceed to fluid-filled pustules. If the patient survives, the blisters turn to scabs that fall off, leaving a pitted scar. Corneal scarring usually results in blindness. The disease is most contagious when the rash is present. Smallpox is usually diagnosed based on the characteristic rash. Microscopic examination of virus particles can also be used, as pox viruses have a unique shape. To conclusively diagnose smallpox and determine the exact strain, the polymerase chain reaction (PCR) can be used to demonstrate the unique DNA “fingerprint” of the virus strain.

Death from smallpox usually occurs during the initial rash. A different strain of Variola major, also known as hemorrhagic smallpox, produces internal bleeding and may cause death prior to rash formation. If a patient survives, he or she will be immune to smallpox for life. This immunity has been known for thousands of years. An early immunization technique known as variolation involved transferring infectious samples (pus or scabs) from a patient with mild smallpox to a healthy person via the nostrils or skin. In the 18th century rural Europe, it was known that milkmaids became immune to smallpox after contracting cowpox from the udders of cows. In 1796, Edward Jenner, an English country doctor, extracted pus from a cowpox-infected milkmaid and injected it into a healthy young boy. He then performed experiments to “challenge” the boy’s skin by variolation with smallpox scabs, which verified that the boy was immune to smallpox. Jenner’s work was the birth of the modern-day vaccination.

It is believed that immunity from vaccination diminishes after about ten years, although there is much disagreement over this subject. In 1952, the World Health Organization (WHO) initiated a worldwide vaccination campaign to eradicate smallpox. This program resulted in the steady decline of the virus with the last naturally occurring case in Somalia in 1977. On May 8, 1980, the WHO declared smallpox as officially eradicated from the world.

Despite eradication of natural smallpox, many experts fear that this disease could be reintroduced into the population intentionally by terrorists. As immunity is thought to diminish after ten years and mass vaccinations ceased in the mid 1970s, the current world population would likely be susceptible to the virus. Since the virus is very stable in aerosol droplets, it is possible that terrorists could employ some type of spraying device to attack an airport or other crowded building. Due to our highly mobile society and the high transmissability of smallpox, one single case of smallpox may constitute a worldwide emergency.

POSSIBLE BIOTERRORISM SCENARIO

Following intelligence from overseas sources, the FBI issues a terrorist alert to the city of Northwest, with a population of 1.5 million. Local authorities are instructed to tighten security at large gatherings such as sporting events and music concerts. Health officials are directed to report any unusual medical cases that could represent bioterrorism.

Twenty days after the alert, a 27-year old Caucasian man appears at a local hospital emergency room with 106-degree fever and pustules concentrated on his face and extremities. At least one member of his family had a similar ailment. Twelve days earlier, the man had attended a football game with over 80,000 people present. In consideration of the terrorist alert, the man is immediately placed in isolation and tested for smallpox. A preliminary antibody-based test, however, is negative. One of the hospital physicians, who had been in Central Africa ten years before, diagnosed the patient’s symptoms to be very similar to cases of monkeypox he had treated. The rest of the attending physicians concurred. In fact, the man’s brother-in-law worked at the local zoo that had recently received several animals from a rainforest in the Democratic Republic of the Congo. To conclusively determine whether the man had smallpox, resulting from a bioattack, or monkeypox, which is much less contagious, fluid is taken from a large pustule on the patient’s arm. This fluid is then immediately sent to the Centers for Disease Control (CDC) in Atlanta for PCR testing.

This experiment is a simulation of molecular biology tests used for the detection of the smallpox virus.
**Experiment Overview**

**EXPERIMENT OBJECTIVE:**

The objective of this experiment is to develop an understanding of Smallpox and the causative agent of the disease. Students will analyze simulated PCR products to confirm or rule out the presence of the Smallpox virus.

**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. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

**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.
Experiment Overview

1. Prepare agarose gel in casting tray.

2. Remove end blocks & comb, then submerge gel under buffer in electrophoresis chamber.

3. Load each sample in consecutive wells.

4. Attach safety cover, connect leads to power source and conduct electrophoresis.

5. After electrophoresis, transfer gel for staining.

6. Analysis on white light source.

Gel pattern will vary depending upon experiment.

DNA SCREENING FOR SMALLPOX

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Module I: Agarose Gel Electrophoresis

CASTING THE AGAROSE GEL

1. **DILUTE** concentrated 50X Electrophoresis buffer with distilled water (refer to Table A for correct volumes depending on the size of your gel casting tray).
2. **MIX** agarose powder with buffer solution in a 250 ml flask (refer to Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60° C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

**Table A**

<table>
<thead>
<tr>
<th>Size of Gel Casting tray</th>
<th>Concentrated Buffer (50x)</th>
<th>Distilled Water</th>
<th>Amount of Agarose</th>
<th>TOTAL Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 x 7 cm</td>
<td>0.6 ml</td>
<td>29.4 ml</td>
<td>0.23 g</td>
<td>30 ml</td>
</tr>
<tr>
<td>7 x 10 cm</td>
<td>1.0 ml</td>
<td>49.0 ml</td>
<td>0.39 g</td>
<td>50 ml</td>
</tr>
<tr>
<td>7 x 14 cm</td>
<td>1.2 ml</td>
<td>58.8 ml</td>
<td>0.46 g</td>
<td>60 ml</td>
</tr>
</tbody>
</table>

**IMPORTANT:**

If you are unfamiliar with agarose gel prep and electrophoresis, detailed instructions and helpful resources are available at www.edvotek.com

**Wear gloves and safety goggles**
Module I: Agarose Gel Electrophoresis

RUNNING THE GEL

8. **PLACE** the gel (still on the tray) into the electrophoresis chamber. **COVER** the gel with 1X Electrophoresis Buffer (See Table B for recommended volumes). The gel should be completely submerged.

9. **PUNCTURE** the foil overlay of the QuickStrip™ with a pipet tip. **LOAD** the entire sample (35 μl) into the well in the order indicated by Table 1, at right.

10. **PLACE** safety cover on the unit. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.

11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines). Allow the tracking dye to migrate at least 3.5 cm from the wells.

12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and proceed to instructions for **STAINING** the agarose gel.

### Table 1: Gel Loading

<table>
<thead>
<tr>
<th>Lane</th>
<th>Tube A</th>
<th>Tube B</th>
<th>Tube C</th>
<th>Tube D</th>
<th>Tube E</th>
<th>Tube F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard DNA Marker</td>
<td>Simulated Smallpox positive control marker</td>
<td>Negative control</td>
<td>Cowpox control DNA sample</td>
<td>Sample from Patient #1</td>
<td>Sample from Patient #2</td>
</tr>
</tbody>
</table>

### Table B: 1x Electrophoresis Buffer (Chamber Buffer)

<table>
<thead>
<tr>
<th>EDVOTEK Model #</th>
<th>Total Volume Required</th>
<th>50x Conc. Buffer</th>
<th>Distilled Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>M6+ &amp; M12 (new)</td>
<td>300 ml</td>
<td>6 ml</td>
<td>294 ml</td>
</tr>
<tr>
<td>M12 (classic)</td>
<td>400 ml</td>
<td>8 ml</td>
<td>392 ml</td>
</tr>
<tr>
<td>M36</td>
<td>1000 ml</td>
<td>20 ml</td>
<td>980 ml</td>
</tr>
</tbody>
</table>

### Table C: Time & Voltage Guidelines (0.8% Agarose Gel)

<table>
<thead>
<tr>
<th>Electrophoresis Model</th>
<th>M6+</th>
<th>M12 (new)</th>
<th>M12 (classic) &amp; M36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volts</td>
<td>Min./Max.</td>
<td>Min./Max.</td>
<td>Min./Max.</td>
</tr>
<tr>
<td>150</td>
<td>15/20 min.</td>
<td>20/30 min.</td>
<td>25 / 35 min.</td>
</tr>
<tr>
<td>125</td>
<td>20/30 min.</td>
<td>30/35 min.</td>
<td>35 / 45 min.</td>
</tr>
<tr>
<td>75</td>
<td>35 / 45 min.</td>
<td>55/70 min.</td>
<td>60 / 90 min.</td>
</tr>
</tbody>
</table>

**REMINDER:** Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.
Module II-A: Staining Agarose Gels Using FlashBlue™

1. **DILUTE** 10 ml of 10x concentrated FlashBlue™ with 90 ml of water in a flask and **MIX** well.
2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray into a small, clean gel-staining tray.
3. **COVER** the gel with the 1x FlashBlue™ stain solution. **STAIN** the gel for 5 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. **STAINING THE GEL FOR LONGER THAN 5 MINUTES WILL REQUIRE EXTRA DESTAINING TIME.**
4. **TRANSFER** the gel to a second small tray. **COVER** the gel with water. **DESTAIN** for at least 20 minutes with gentle shaking (longer periods will yield better results). Frequent changes of the water will accelerate destaining.
5. Carefully **REMOVE** the gel from the destaining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

**ALTERNATIVE PROTOCOL:**

1. **DILUTE** one ml of concentrated FlashBlue™ stain with 149 ml dH₂O.
2. **COVER** the gel with diluted FlashBlue™ stain.
3. **SOAK** the gel in the staining liquid for at least three hours. For best results, stain gels overnight.
Module II-B: Staining Agarose Gels Using InstaStain® Blue

1. Carefully REMOVE the agarose gel and casting tray from the electrophoresis chamber. SLIDE the gel off of the casting tray on to a piece of plastic wrap on a flat surface.
2. MOISTEN the gel with a few drops of electrophoresis buffer.
3. Wearing gloves, PLACE the blue side of the InstaStain® Blue card on the gel.
4. With a gloved hand, REMOVE air bubbles between the card and the gel by firmly running your fingers over the entire surface. Otherwise, those regions will not stain.
5. PLACE the casting tray on top of the gel/card stack. PLACE a small weight (i.e. an empty glass beaker) on top of the casting tray. This ensures that the InstaStain® Blue card is in direct contact with the gel surface. STAIN the gel for 10 minutes.
6. REMOVE the InstaStain® Blue card. If the color of the gel appears very light, reapply the InstaStain® Blue card to the gel for an additional five minutes.
7. TRANSFER the gel to a small, clean gel-staining tray. COVER the gel with about 75 mL of distilled water and DESTAIN for at least 20 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. To accelerate destaining, warm the distilled water to 37°C and change it frequently.
8. Carefully REMOVE the gel from the destaining liquid. VISUALIZE results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

ALTERNATIVE PROTOCOL:

1. Carefully SLIDE the agarose gel from its casting tray into a small, clean tray containing about 75 mL of distilled/deionized water or used electrophoresis buffer. The gel should be completely submerged.
2. Gently FLOAT the InstaStain® Blue card(s) on top of the liquid with the stain (blue side) facing toward the gel. Each InstaStain® Blue card will stain 49 cm² of gel (7 x 7 cm).
3. COVER the tray with plastic wrap to prevent evaporation. SOAK the gel in the staining liquid for at least 3 hours. The gel can remain in the liquid overnight if necessary.
4. Carefully REMOVE the gel from the staining tray. VISUALIZE results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.
Study Questions

1. Why is smallpox such a serious concern?

2. Describe the usual mechanism of smallpox transmission. What are some of the ways in which smallpox was used as a biological weapon?

3. List the symptoms associated with smallpox infection.

4. How was immunity to smallpox first discovered?

5. What are the differences between smallpox, chickenpox, and monkeypox?
## Instructor's Guide

### ADVANCE PREPARATION:

<table>
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<th>Preparation for:</th>
<th>What to do:</th>
<th>When?</th>
<th>Time Required:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Module I:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agarose Gel Electrophoresis</td>
<td>Prepare QuickStrips™</td>
<td>Up to one day before performing the experiment</td>
<td>45 min.</td>
</tr>
<tr>
<td></td>
<td>Prepare diluted electrophoresis buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prepare molten agarose and pour gels</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Module II:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staining Agarose Gels</td>
<td>Prepare staining components</td>
<td>The class period or overnight after the class period</td>
<td>10 min.</td>
</tr>
</tbody>
</table>

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Pre-Lab Preparations: Module I

AGAROSE GEL ELECTROPHORESIS

This experiment requires a 0.8% agarose gel per student group. You can choose whether to prepare the gels in advance or have the students prepare their own. Allow approximately 30-40 minutes for this procedure.

Individual Gel Preparation:

Each student group can be responsible for casting their own individual gel prior to conducting the experiment. See Module I in the Student’s Experimental Procedure. Students will need 50x concentrated buffer, distilled water and agarose powder.

Batch Gel Preparation:

To save time, a larger quantity of agarose solution can be prepared for sharing by the class. Electrophoresis buffer can also be prepared in bulk. See Appendix B.

Preparing Gels in Advance:

Gels may be prepared ahead and stored for later use. Solidified gels can be stored under buffer in the refrigerator for up to 2 weeks.

Do not freeze gels at -20°C as freezing will destroy the gels.

Gels that have been removed from their trays for storage should be “anchored” back to the tray with a few drops of molten agarose before being placed into the tray. This will prevent the gels from sliding around in the trays and the chambers.

SAMPLES FORMAT: PREPARING THE QUICKSTRIPS™

QuickStrip™ tubes consist of a microtiter block covered with a protective overlay. Each well contains pre- aliquoted DNA.

Using sharp scissors, carefully divide the block of tubes into individual strips by cutting between the rows (see diagram at right). Take care not to damage the protective overlay while separating the samples.

Each lab group will receive one set of tubes. Before loading the gel, remind students to tap the tubes to collect the sample at the bottom of the tube.

If using SYBR® Safe or InstaStain® Ethidium Bromide for DNA visualization, each QuickStrip™ is shared by two groups. 18 μl of the DNA sample will be loaded into each well. Proceed to visualize the results as specified by the DNA stain literature.
Pre-Lab Preparations: Module II

**MODULE II-A: STAINING WITH INSTASTAIN® BLUE**

The easiest and most convenient DNA stain available is InstaStain® Blue. InstaStain® Blue does not require the formulation, storage and disposal of large volumes of liquid stain. Each InstaStain® Blue card contains a small amount of blue DNA stain. When the card is placed in water, the DNA stain is released. This solution simultaneously stains and destains the gel, providing uniform gel staining with minimal liquid waste and mess.

You can use a White Light Visualization System (Cat. #552) to visualize gels stained with InstaStain® Blue.

**MODULE II-B: STAINING WITH FLASHBLUE™**

FlashBlue™ stain is optimized to shorten the time required for both staining and destaining steps. Agarose gels can be stained with diluted FlashBlue™ for 5 minutes and destained for only 20 minutes. For the best results, leave the gel in liquid overnight. This will allow the stained gel to “equilibrate” in the destaining solution, resulting in dark blue DNA bands contrasting against a uniformly light blue background. A white light box (Cat. #552) is recommended for visualizing gels stained with FlashBlue™.

- Stained gels may be stored in destaining liquid for several weeks with refrigeration, although the bands may fade with time. If this happens, re-stain the gel.
- Destained gels can be discarded in solid waste disposal. Destaining solutions can be disposed of down the drain.

**MODULE II: PHOTODOCUMENTATION OF DNA (OPTIONAL)**

Once gels are stained, you may wish to photograph your results. There are many different photodocumentation systems available, including digital systems that are interfaced directly with computers. Specific instructions will vary depending upon the type of photodocumentation system you are using.
Experiment Results and Analysis

In the idealized schematic, the relative positions of DNA fragments are shown but are not depicted to scale.

<table>
<thead>
<tr>
<th>Lane</th>
<th>Tube</th>
<th>Sample</th>
<th>Molecular Weights (in bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>DNA Standard Markers</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>Simulated Smallpox Positive Control</td>
<td>3000</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>Negative Control</td>
<td>No Bands</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>Cowpox Control DNA Sample</td>
<td>4282</td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td>Sample from Patient PCR #1</td>
<td>4282</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>Sample from Patient PCR #2</td>
<td>4282</td>
</tr>
</tbody>
</table>

Conclusion: The patient does not have smallpox. It appears that the patient is infected with cowpox.

- Samples E and F are duplicate PCR experiments that yield the same results.
- Sample C is the negative control using a bacterial plasmid. Since the plasmid does not contain viral sequences (specifically, the smallpox virus) no PCR amplification occurred.
Please refer to the kit insert for the Answers to Study Questions
Appendices

A  EDVOTEK® Troubleshooting Guide
B  Bulk Preparation of Agarose Gels
C  Data Analysis Using a Standard Curve

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

**EDVOTEK® Troubleshooting Guides**

<table>
<thead>
<tr>
<th>PROBLEM:</th>
<th>CAUSE:</th>
<th>ANSWER:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bands are not visible on the gel.</td>
<td>The gel was not prepared properly.</td>
<td>Ensure that the electrophoresis buffer was correctly diluted.</td>
</tr>
<tr>
<td></td>
<td>The gel was not stained properly.</td>
<td>Repeat staining.</td>
</tr>
<tr>
<td></td>
<td>Malfunctioning electrophoresis unit or power source.</td>
<td>Contact the manufacturer of the electrophoresis unit or power source.</td>
</tr>
<tr>
<td>After staining the gel, the DNA bands are faint.</td>
<td>The gel was not stained for a sufficient period of time.</td>
<td>Repeat staining protocol.</td>
</tr>
<tr>
<td></td>
<td>The background of gel is too dark.</td>
<td>Destain the gel for 5-10 minutes in distilled water.</td>
</tr>
<tr>
<td>DNA bands were not resolved.</td>
<td>Tracking dye should migrate at least 3.5 cm (if using a 7x7 cm tray), and at least 6 cm (if using a 7x14 cm tray) from the wells to ensure adequate separation.</td>
<td>Be sure to run the gel at least 6 cm before staining and visualizing the DNA (approximately one hour at 125 V).</td>
</tr>
<tr>
<td>DNA bands fade when gels are kept at 4°C.</td>
<td>DNA stained with FlashBlue™ may fade with time</td>
<td>Re-stain the gel with FlashBlue™</td>
</tr>
<tr>
<td>There is no separation between DNA bands, even though the tracking dye ran the appropriate distance.</td>
<td>The wrong percent gel was used for electrophoretic separation.</td>
<td>Be sure to prepare the correct percent agarose gel. For reference, the Ready-to-Load™ DNA samples should be analyzed using a 0.8% agarose gel.</td>
</tr>
<tr>
<td>There’s not enough sample in my QuickStrip™.</td>
<td>The QuickStrip™ has dried out.</td>
<td>Add 40 µL water, gently pipet up and down to mix before loading.</td>
</tr>
</tbody>
</table>
Appendix B

Bulk Preparation of Agarose Gels

To save time, the electrophoresis buffer and agarose gel solution can be prepared in larger quantities for sharing by the class. Unused diluted buffer can be used at a later time and solidified agarose gel solution can be remelted.

**Bulk Electrophoresis Buffer**

Quantity (bulk) preparation for 3 liters of 1x electrophoresis buffer is outlined in Table D.

<table>
<thead>
<tr>
<th>Amt of Buffer</th>
<th>Concentrated Buffer (50X)</th>
<th>Distilled Water (ml)</th>
<th>Total Volume Required (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 ml</td>
<td>2,940 ml</td>
<td></td>
<td>3000 ml (3 L)</td>
</tr>
</tbody>
</table>

**Batch Agarose Gels (0.8%)**

For quantity (batch) preparation of 0.8% agarose gels, see Table E.

1. Use a 500 ml flask to prepare the diluted gel buffer.
2. Pour 3.0 grams of UltraSpec-Agarose™ into the prepared buffer. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.
4. Heat the agarose solution as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 60°C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.
6. Dispense the required volume of cooled agarose solution for casting each gel. Measure 30 ml for a 7 x 7 cm tray, 50 ml for a 7 x 10 cm tray, and 60 ml for a 7 x 14 cm tray. For this experiment, 7 x 7 cm gels are recommended.
7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Then proceed with preparing the gel for electrophoresis.
Appendix C
Data Analysis Using a Standard Curve

Agarose gel electrophoresis separates biomolecules into discrete bands, each comprising molecules of the same size. How can these results be used to determine the lengths of different fragments? Remember, as the length of a biomolecule increases, the distance to which the molecule can migrate decreases because large molecules cannot pass through the channels in the gel with ease. Therefore, the migration rate is inversely proportional to the length of the molecules—more specifically, to the \( \log_{10} \) of molecule's length. To illustrate this, we ran a sample that contains bands of known lengths called a “standard”. We will measure the distance that each of these bands traveled to create a graph, known as a “standard curve”, which can then be used to extrapolate the size of unknown molecule(s).

1. Measure and Record Migration Distances

Measure the distance traveled by each Standard DNA Fragment from the lower edge of the sample well to the lower end of each band. Record the distance in centimeters (to the nearest millimeter) in your notebook. Repeat this for each DNA fragment in the standard.

Measure and record the migration distances of each of the fragments in the unknown samples in the same way you measured the standard bands.

2. Generate a Standard Curve.

Because migration rate is inversely proportional to the \( \log_{10} \) of band length, plotting the data as a semi-log plot will produce a straight line and allow us to analyze an exponential range of fragment sizes. You will notice that the vertical axis of the semi-log plot appears atypical at first; the distance between numbers shrinks as the axis progresses from 1 to 9. This is because the axis represents a logarithmic scale. The first cycle on the y-axis corresponds to lengths from 100-1,000 base pairs, the second cycle measures 1,000-10,000 base pairs, and so on. To create a standard curve on the semi-log paper, plot the distance each Standard DNA fragment migrated on the x-axis (in mm) versus its size on the y-axis (in base pairs). Be sure to label the axes!
Appendix C

Data Analysis Using a Standard Curve

After all the points have been plotted, use a ruler or a straight edge to draw the best straight line possible through the points. The line should have approximately equal numbers of points scattered on each side of the line. It is okay if the line runs through some points (see Figure 3 for an example).

3. Determine the length of each unknown fragment.
   
a. Locate the migration distance of the unknown fragment on the x-axis of your semi-log graph. Draw a vertical line extending from that point until it intersects the line of your standard curve.

b. From the point of intersection, draw a second line, this time horizontally, toward the y-axis. The value at which this line intersects the y-axis represents the approximate size of the fragment in base pairs (refer to Figure 3 for an example). Make note of this in your lab notebook.

c. Repeat for each fragment in your unknown sample.
## Appendix C

<table>
<thead>
<tr>
<th>Y-axis: Base Pairs</th>
<th>10,000</th>
<th>9,000</th>
<th>8,000</th>
<th>7,000</th>
<th>6,000</th>
<th>5,000</th>
<th>4,000</th>
<th>3,000</th>
<th>2,000</th>
<th>1,000</th>
<th>900</th>
<th>800</th>
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<th>60</th>
<th>50</th>
<th>40</th>
<th>30</th>
<th>20</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-axis: Migration distance (cm)</td>
<td>1 cm</td>
<td>2 cm</td>
<td>3 cm</td>
<td>4 cm</td>
<td>5 cm</td>
<td>6 cm</td>
<td></td>
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