The Fungus Among Us: Valley Fever in California MiniLab

Student Guide

Cat# M3020

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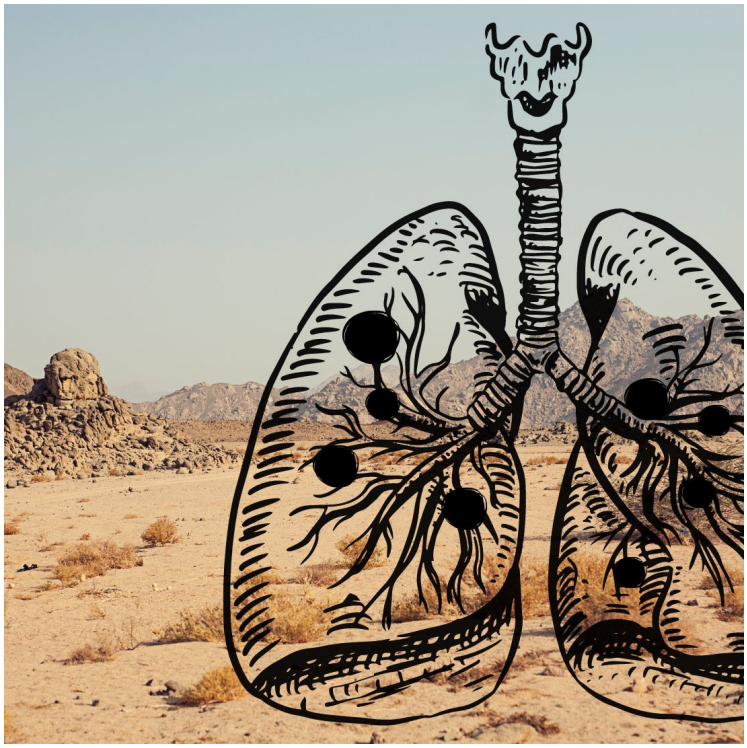

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Laboratory Safety

1. Wear lab coats, gloves, and eye protection as required by district protocol.
2. Use caution with all electrical equipment such as PCR machines and electrophoresis units.
3. Heating and pouring molten agarose is a splash hazard. Use caution when handling hot liquids. Wear eye protection and gloves to prevent burns.
4. Wash your hands thoroughly after handling biological materials and chemicals.

Day 1 - Introduction

Have you ever heard of Valley Fever? Depending on where you live, this is a disease that might not be on your radar as yet. However, it is something that anyone living in or visiting certain areas in the southwestern part of the United States should be aware of. In this MiniLab, we will delve into the fascinating and sometimes frightening world of Valley Fever, explore its causes, symptoms, diagnosis, risk factors, preventative measures, and simulate a laboratory test to detect the presence of the disease-causing fungus in soil samples.

What are prokaryotes and eukaryotes?

All living things are classified into two main categories: prokaryotes and eukaryotes. Prokaryotes, which include kingdom bacteria and archaea, are characterized by the absence of a true nucleus and other membrane-bound organelles. The genetic material in prokaryotes is present as a circular chromosome, and the cells are generally smaller and simpler. Prokaryotes reproduce asexually by binary fission. On the other hand, eukaryotes, which include animals, plants, protists, and fungi, possess a distinct nucleus enclosed by a nuclear membrane and various membrane-bound organelles, such as mitochondria and endoplasmic reticulum. Eukaryotic cells are typically larger, more complex, and undergo mitosis or meiosis for reproduction.

What are fungi?

Figure 1: Image of various types of fungi (https://en.wikipedia.org/wiki/Fungus)

Fungi are a group of eukaryotic organisms that range from microscopic yeasts to towering mushrooms and are found in a variety of habitats, from forests and grasslands to human environments, including your dinner table. Like humans, fungi are heterotrophs, meaning they are not capable of producing their own food. However, unlike humans who have to consume other organisms or their byproducts to obtain our nutrients, fungi obtain nutrients by secreting

enzymes into their surroundings to break down organic matter and absorbing the resulting nutrients, known as external digestion. Fungi exist as single cells or multicellular structures, have diverse life cycles, and can be found in just about any kind of environment, but are particularly common in soils or on plant material.

Fungi play a crucial role in the environment and food web, functioning as nature's recyclers and symbiotic partners. One of the primary benefits of fungi lies in their role as decomposers, or nature's clean-up crew, breaking down organic matter into essential nutrients. This process, known as litter decomposition, releases carbon, nitrogen, and phosphorus back into the soil, providing a vital source of nutrition for new plant growth. Additionally, fungi form symbiotic relationships with roots of plants, called mycorrhizae. The term "mycorrhiza" is derived from the Greek words "mycos," meaning fungus, and "rhiza," meaning root. In these partnerships, fungi assist plants in absorbing water and nutrients from the soil, while plants provide fungi with sugars produced through photosynthesis. This mutualistic exchange of resources benefits both partners, the plant gains improved access to essential nutrients, and the fungus receives a source of energy. This thus enhances the overall health and resilience of both fungi and plants, illustrating the interconnectedness of life in the food web.

While fungi provide numerous benefits, including medicine (antibiotics, immunosuppressants), food production (edible mushrooms, fermentation), environmental cleanup, research and industry (model organisms, enzyme production), biotechnology (drug and biofuel production), and agriculture (mycorrhizal associations, biopesticides), certain species can also pose risks and challenges. Some fungi act as pathogens, causing diseases in plants, animals, and humans. Plant diseases caused by fungi can lead to significant losses in agricultural productivity. Mold growth in indoor environments poses another set of challenges, leading to structural damage and potential health risks, primarily as allergens. Some molds produce mycotoxins, harmful substances that can contaminate food supplies, posing a risk to both human and animal health. On the human front, fungal infections can range from minor skin rashes to severe systemic illnesses. Additionally, certain fungi produce allergens that can trigger respiratory issues like asthma or allergic sinusitis in sensitive individuals. Balancing the appreciation for the positive contributions of fungi with awareness of their potential harmful aspects is crucial for effectively managing their impact on ecosystems and human activities.

What is Valley Fever?

Valley Fever, also known as coccidioidomycosis, [kok-sid-ee-oi-doh-mahy-koh-sis] is a fungal infection caused by two species of fungi: *Coccidioides immitis* and *Coccidioides posadasii*. The name "Valley Fever" originates from its discovery in the San Joaquin Valley of California, although it can be contracted in various other areas with similar environmental conditions. These fungi primarily thrive in the soil of arid and semi-arid regions, and have been found in California, Utah, Washington, Nevada, Arizona, New Mexico, Texas, Mexico, and Central and South America (see map below), with the highest prevalence found in San Joaquin Valley in California and in the Sonoran Desert of Arizona. According to the Centers for Disease Control (CDC), the distribution of this fungus might be changing as environmental conditions change.

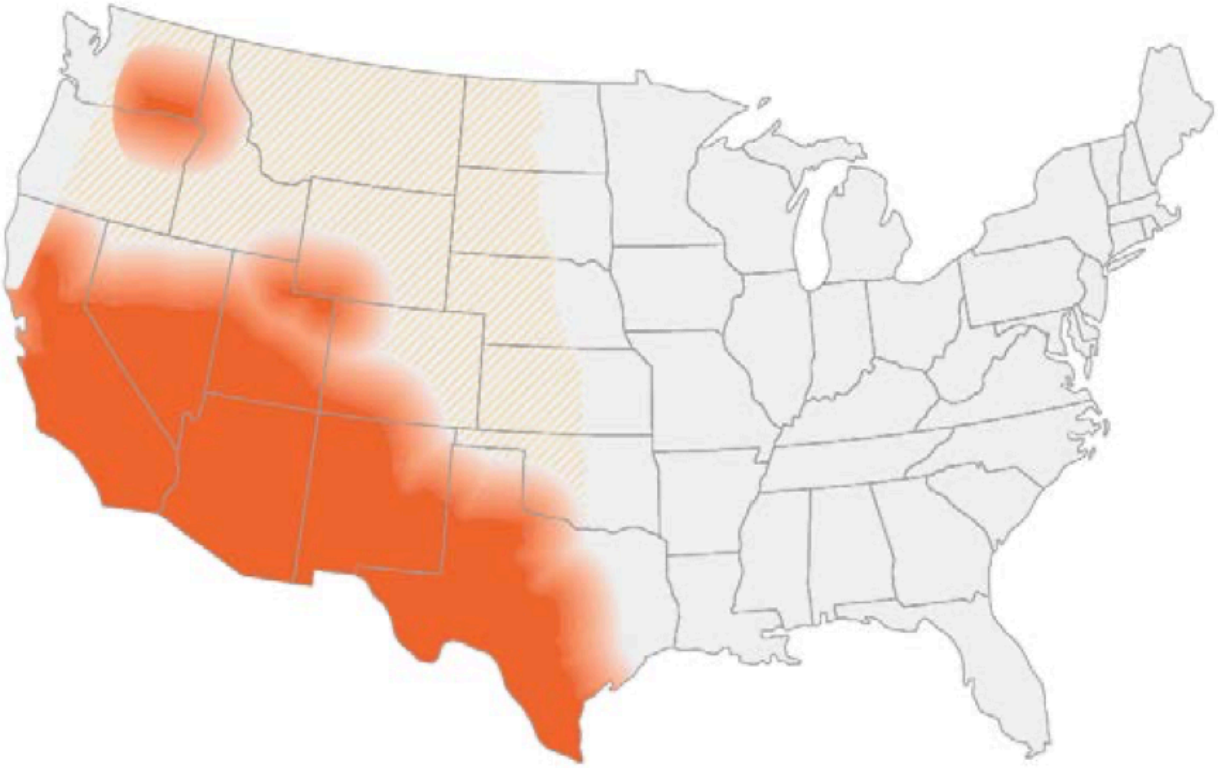


Figure 2: Map showing the areas that are endemic for Coccidioidomycosis in the US.
https://www.cdc.gov/fungal/pdf/more-information-about-fungal-maps-508.pdf

Valley Fever is caused when individuals inhale spores from the *Coccidioides* species of fungi that that are present in the soil and dirt. Disturbing the soil or dirt, whether through activities like construction, farming, or even natural events like dust storms, can lead to the release of these spores into the air that can be inhaled by both humans and animals without knowing it. These dispersed spores may remain airborne for long periods of time and potentially travel over long distances. Most people who breathe in the spores do not become sick, but a small percentage who do may then develop Valley Fever.

Anyone residing in or traveling to regions where the *Coccidioides* fungi are present is at risk of contracting Valley Fever. Of the diagnosed Valley fever cases in the United States, about 60% of cases occur in Arizona and 30% in California, and the remaining 10% are dispersed in other areas. Valley fever can be contracted throughout the year, but there is a higher likelihood of individuals becoming infected with the fungus responsible for Valley fever during late summer and fall compared to other seasons.

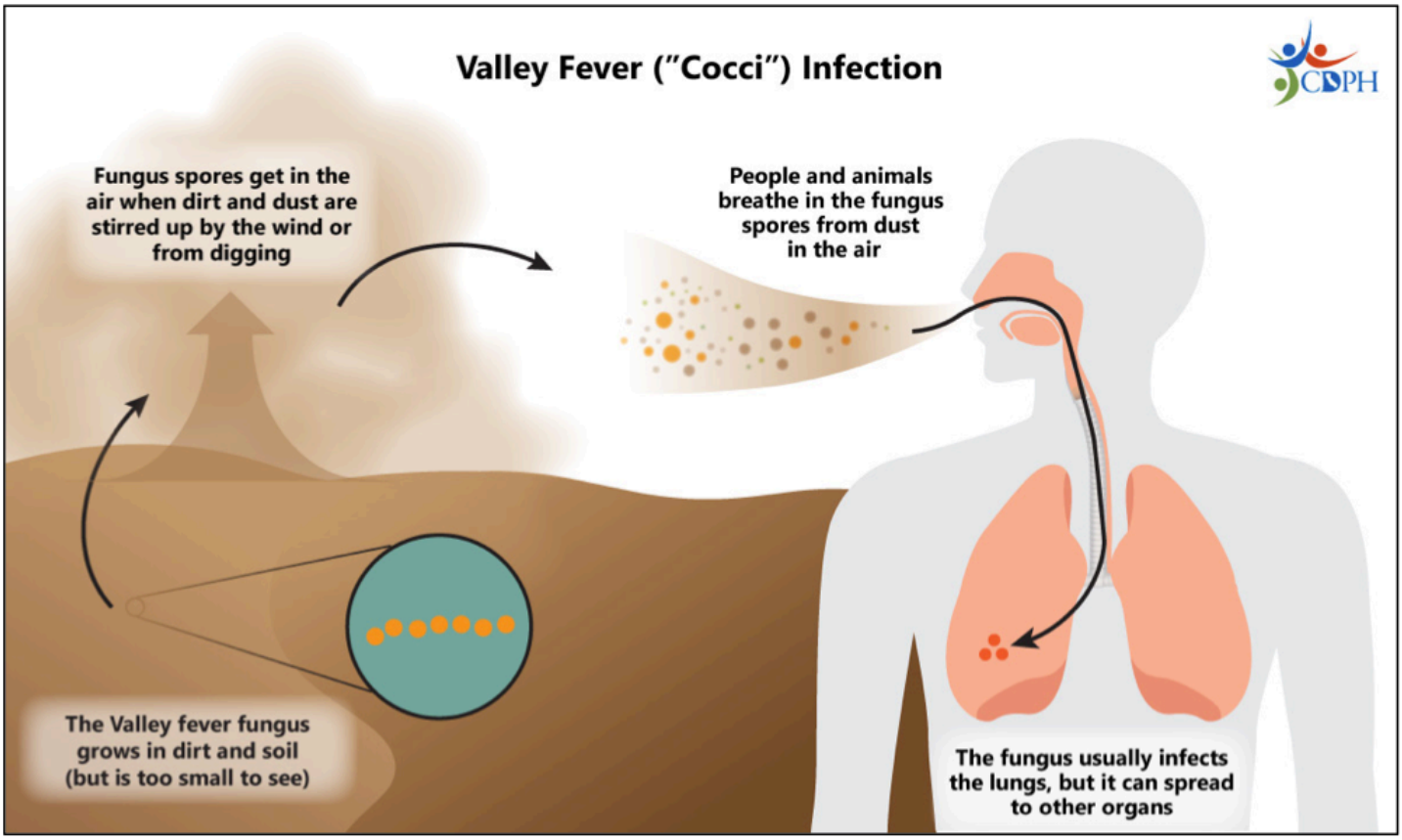


Figure 3: Infographic of Valley Fever Infection Route
https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/ValleyFeverFactSheet.pdf

The signs and symptoms of Valley Fever typically appear one to three weeks after exposure and can range from mild to severe. Common symptoms include fever, cough, fatigue, shortness of breath, and chest pain, all due to the pneumonia that is caused by these fungi. Some people also develop joint and muscle pain, and skin rashes, which are part of the body's immune response to this fungus. In severe cases, the infection can spread to other parts of the body, leading to more serious symptoms and complications such as bone or joint infections, meningitis, and skin or soft tissue abscesses. When the infection causes disease outside the chest that is known as disseminated Valley Fever.

Misdiagnosis of Coccidioidomycosis sometimes occurs because the initial symptoms of Valley Fever, such as fever, cough, and fatigue, can be non-specific and easily mistaken for other respiratory infections, such as the flu or pneumonia. Furthermore, because Coccidioidomycosis is more prevalent in specific geographic areas, healthcare providers in regions where the disease is rare may not consider it as a potential diagnosis, and can contribute to delays in diagnosis or a misdiagnosis. Several diagnostic tests are available for Coccidioidomycosis, including *Coccidioides* antibody testing, fungal culture, nucleic acid testing, chest x-ray or imaging studies, and skin testing. The choice of tests used depends on various factors, including the stage of the infection, the severity of the symptoms, and the patient's overall health.

Some individuals are at a higher risk of developing severe disease, including those with weakened immune systems, pregnant women in their last trimester, people with diabetes, older

adults, and certain ethnic groups, particularly those who are of African or Filipino ancestry. Determining why some ethnic groups are more susceptible to developing severe disease is an active area of research, but it is thought that certain people may be genetically predisposed. In recent studies in mouse models, researchers found that mice with a mutation in the CLEC7A gene, which encodes for the Dectin-1 protein, are more susceptible to infection by *Coccidioides*. Dectin-1 is part of the innate immune system. Dectin-1 is a protein expressed on the surface of many immune cells that recognizes beta-glucans, which are part of the walls of many fungi. Dectin-1 protein is responsible for activating an immune response pathway against *Coccidioides.* Interestingly, in a small observational study of humans with severe disseminated Valley Fever, all East Asians in the study cohort expressed the mutated form of Dectin-1, suggesting that Dectin-1 is an important determinant of whether an individual has a higher potential to experience severe Valley Fever disease. Additional protein-encoding genes involved in the immune response pathway against *Coccidioides*, including PLCG2, DUOX1, and DUOXA1 are promising targets for understanding why certain individuals experience more severe Valley Fever disease. Preliminary studies of these genes suggest that they play a role in the innate fungal recognition process, which results in impaired or delayed immune responses. This delay permits the fungus to rapidly multiply and establish itself in extra-thoracic sites while effectively evading the effects of the body's immune system.

Preventative measures include staying indoors during dust storms, avoiding outdoor activities in dusty areas, such as construction or excavation sites, using dust masks while outdoors, keeping skin injuries clean with soap and water, and taking antifungal medications as prescribed by a healthcare professional. The good news is that Valley Fever is not contagious and cannot spread from person to person, and a lifetime immunity is usually acquired, meaning you cannot get Valley Fever again after you have been infected the first time. The bad news is that there is no vaccine to prevent Valley Fever. Currently, there is ongoing research at the University of Arizona to develop a vaccine for Valley Fever in dogs, based on a mutant of *C. posadasii*, that was shown to not cause disease in several strains of immunosuppressed mice and in healthy dogs, but it does immunize them so that they resist infection with a virulent fungus. This promising study might create a pathway towards developing a vaccine to prevent Valley Fever in humans.

If you or someone you know suspect that they may have Valley Fever, or if symptoms manifest after exposure in a high-risk area, it's crucial to seek medical attention for diagnosis and appropriate treatment. The diagnosis is usually made with a blood test for antibodies against proteins made by the Coccidioides fungi. It takes a few weeks after exposure to the fungus before the tests are able to detect antibodies against the fungus. Sometimes the fungus can be grown from a sample of infected tissue, or the fungus can be seen under the microscope in the removed tissue. Because the tissue form of Coccidioides is so unusual, it will not be confused with other fungi that might cause similar infections.

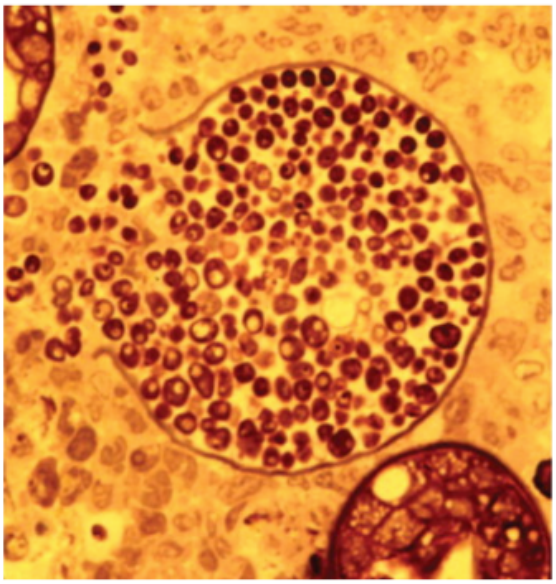


Figure 4. *Coccidioides immitis* can be found in lungs as a large spherule containing endospores. These spherules can rupture and endospores can spill into the surrounding lung where they mature into new spherules.
https://www.sciencedirect.com/science/article/abs/pii/B978012373944500184X

Valley Fever can usually be managed with antifungal drugs from the "Azole" family, and most individuals recover fully. The length of treatment usually depends on the severity of symptoms and disease, and complications of the disease. Some patients take antifungal medications for a few months, while others may need lifetime therapy to control Valley Fever. More severe cases may necessitate hospitalization for more intensive care. Some people recover from pneumonia without treatment. In any case, people who recover from infection are immune to reinfection.

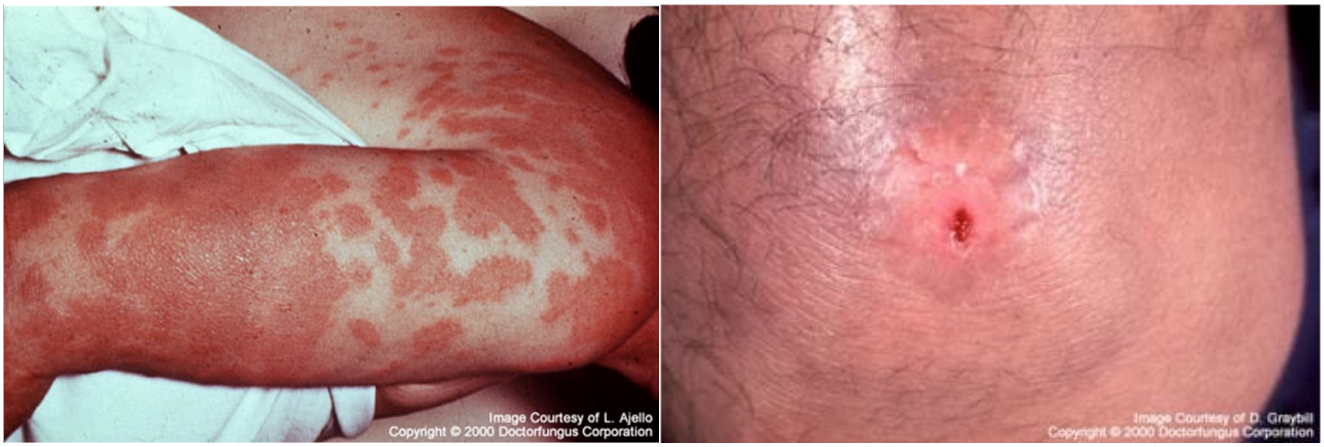


Figure 5 - Cutaneous displays of cocciodiomycosis
https://www.merckmanuals.com/home/infections/fungal-infections/coccidioidomycosis

The Fungus Life Cycle

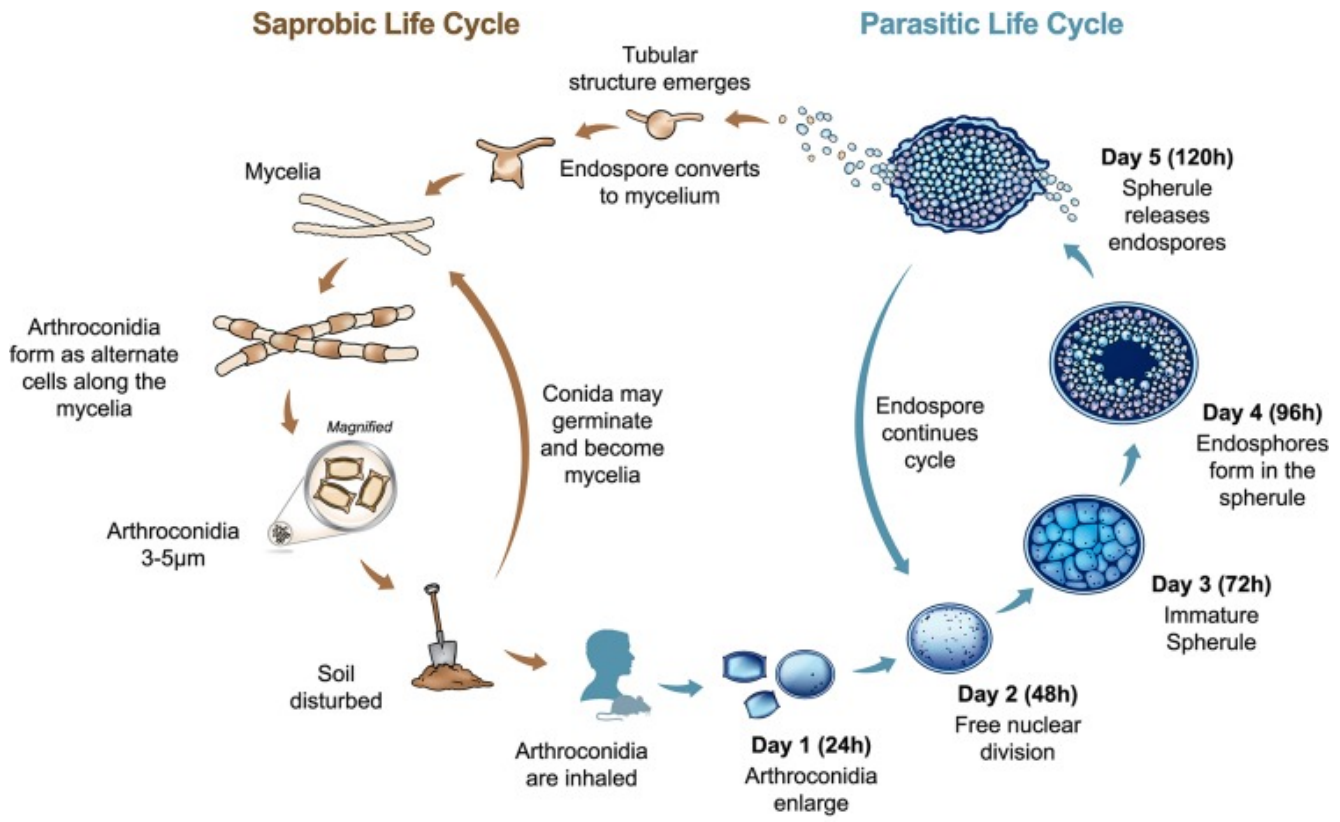


Figure 6 - Life cycle of *Cocciodiodes*
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4431877/figure/ppat.1004762.g001/

The *Coccidioides* fungus is able to switch between two life cycles, saprobic and parasitic, depending on the environment in which it lives. *Coccidioides* fungus are found in the **saprobic life cycle** when they are present in the soil or dirt. In this environment, where moisture is limited, the fungus grows as mycelia, which are vegetative, thread-like structures of fungi. Arthroconidia, which are specialized asexual reproductive structures, form as single-celled barrel-shaped conidia (or spores). These spores serve as a means of dispersal for the fungus, laying in wait in the dirt or soil to be inhaled by a human or animal.

The **parasitic life cycle** takes place when the fungal spores are inhaled and end up in the lungs of their host. Once the arthroconidia (or spores) enter a person's lungs, the warm, moist CO_2 rich environment allows the spores to grow into spherules, which then break open and release small endospores that can spread throughout the lungs or to other parts of the body. These endospores can then transform into spherules, initiating a continuous cycle that leads to an exponential rise in the number of organisms in the lungs.

Pre-Lab Questions

1. Compare prokaryotes and eukaryotes. Fill in the T-Chart below.

Prokaryotes	Eukaryotes

2. Compare the fungal mode of obtaining nutrients to the way humans obtain nutrients.

3. Describe the mutualistic relationship between fungi and plants. Provide one example of a mutualistic relationship that exists between two other organisms.

4. How is Valley Fever contracted, and what can be done to decrease the risk of getting Valley Fever?

5. What four genes have been identified as potential targets to understanding disseminated Valley Fever, and what role do researchers think these genes play in the disease cycle? Which two subpopulations have higher risk of developing disseminated Valley Fever?

6. Name and describe the two life cycles of *Coccidioides* fungi.

Case Studies

Case Study 1: Maria	Case Study 2: Tyrone	Case Study 3: Isabella
Age: Ethnicity: Location: How was Valley Fever contracted: Symptoms: Result: Other Notes:	Age: Ethnicity: Location: How was Valley Fever contracted: Symptoms: Result: Other Notes:	Age: Ethnicity: Location: How was Valley Fever contracted: Symptoms: Result: Other Notes:

I notice, I wonder Graphic Organizer

I notice...	I wonder...

Case Study 1: Maria

Maria, an adventurous 25-year-old Filipino-American, had recently relocated to Phoenix, Arizona, for an exciting job opportunity in the sunny desert. She was filled with youthful enthusiasm for her new surroundings, embracing the Arizona landscape with open arms. On weekends, she embarked on hiking expeditions into the rugged terrain of the Superstition Mountains, eager to explore the hidden treasures of the desert.

On one of these adventurous outings, Maria ventured deep into the heart of the desert, where her footsteps stirred up clouds of fine, dusty soil. Unbeknownst to her, with every breath, she was inhaling the microscopic spores of the *Coccidioides* fungi, setting the stage for a formidable battle ahead.

Two weeks later, her vibrant life took an unexpected turn. A persistent, hacking cough plagued her, fatigue seemed to smother her spirit, and her temperature soared to feverish heights. At first, she brushed it off as a minor ailment, attributing her symptoms to the common cold or perhaps the flu. But as her health continued to deteriorate, she couldn't ignore the relentless cough, which seemed as unrelenting as her youthful determination to conquer the wilderness.

Seeking relief and answers, Maria visited a local clinic, where doctors initially misdiagnosed her condition as a bacterial lung infection due to her persistent cough. With a prescription for antibiotics in hand, she returned home, hopeful that her suffering would soon end.

However, another three weeks passed,, and Maria's condition did not improve. In fact, her symptoms worsened. Breathing became a painful ordeal, and her skin lost its radiant glow, taking on an ashen pallor. The realization that something far more serious was at play dawned on her, and she returned to the clinic with growing concern.

It was at this moment that a vigilant doctor recognized the urgency of her case, promptly ordering a blood test for Valley Fever. The misdiagnosis had cost Maria valuable time, allowing the fungal infection to progress unchecked. The diagnosis, once received, was a mixed blessing - Valley Fever was the culprit behind her suffering, and now it could be treated. The bad news was that her case was alarmingly severe, with the fungal infection having infiltrated her lungs and disseminated to other parts of her body.

The constant cough turned into severe fits, each breath a painful reminder of the hidden battle in her lungs. Fatigue weighed on her heavily, stifling her once energetic spirit. Even standing became a difficult task, and the healthy glow vanished, leaving a pale complexion. As her symptoms worsened, her determination to conquer the wilderness now shifted to a fierce determination to overcome the invisible foe that had taken residence within her.

Maria embarked on a long and challenging journey to recovery, marked by multiple hospital stays, intensive antifungal treatment, and months of rehabilitation. Her story stands as proof of resilience, a living testament to the strength to persevere against the most severe form of Valley Fever. Throughout the arduous process, Maria found strength in unexpected places. The support of friends and family became a crucial pillar, and her own resilience emerged as a beacon of hope. Maria's experience served as a stark reminder of the hidden dangers beneath the desert's surface, not just for herself but for all who ventured into these arid landscapes, carrying a message of awareness and hope for all who would listen.

Case Study 2: Tyrone

Tyrone, a 50-year-old African-American man, had spent his entire life in Bakersfield, California, tending to his garden, earning a reputation for his green thumb in the neighborhood. Little did he know that his therapeutic connection with the soil would expose him to a silent threat. The soil harbored a microscopic menace – fungal spores that gave rise to a silent adversary known as Valley Fever.

As Tyrone devoted countless hours to his garden, cultivating life from the earth, he unknowingly disturbed the soil, releasing fungal spores into the air. These spores silently infiltrated Tyrone's respiratory system, setting the stage for a series of insidious symptoms. Over several months, subtle changes took hold— a persistent cough, chest pain, and an unexplained weight loss, silently eroding his once-vibrant spirit.

Growing alarmed by his deteriorating health, Tyrone decided to seek medical attention, recognizing that his symptoms were far from ordinary. The doctor's office became a stark contrast to the serenity of his garden, and the concern etched across his face mirrored the internal struggle he faced.

Initially, the doctors suspected tuberculosis, noting damage to his lungs on chest x-rays. Antibiotic treatment was initiated, with the hope of putting an end to Tyrone's suffering. However, as the weeks passed, it became evident that the prescribed remedy was ineffective. The persistent assault on Tyrone's well-being prompted a deeper investigation into his condition.

Further tests unraveled the mystery, revealing a different and equally challenging diagnosis: Valley Fever. Scans provided a detailed view of the extent of lung involvement, showing the intricate patterns of fungal infiltration. The severity of the infection became palpable, underscoring the formidable challenge Tyrone now confronted. What made his situation even more challenging was his African-American heritage, which placed him at a higher risk for a more severe manifestation of the disease. Studies have shown that individuals of African or Filipino descent are more likely to experience severe cases of Valley Fever, a factor that added an additional layer of complexity to Tyrone's battle.

The diagnosis not only explained his symptoms but also set the stage for a demanding journey to recovery. Tyrone faced months of antifungal treatment and careful monitoring, with his healthcare team considering the nuances associated with his heritage to tailor the approach to his specific needs. Tyrone faced persistent side effects from Valley Fever during his prolonged recovery. The antifungal treatment brought fatigue, nausea, and skin rashes, challenging his endurance. The slow progress involved monitoring lung improvement through scans, with signs of fading fungal patterns offering hope amid ongoing challenges.

Daily life became a series of milestones, highlighting the resilience needed in Tyrone's battle. While the side effects persisted,Tyrone's unwavering dedication, supported by his healthcare team, family, and friends, showcased his enduring effort to overcome the challenges he faced. Tyrone's story became an inspiration for those facing extended recoveries, emphasizing the importance of patience, support, and an unyielding spirit. His enduring journey highlighted the complexities of overcoming Valley Fever, demonstrating that recovery demands time, care, and a determined will.

Case Study 3: Isabella

Isabella, a 17-year-old high school student and a Caucasian American girl, found herself deeply enamored with the beauty of California's Central Valley. Nestled between the Sierra Nevada mountain range and the coastal ranges, this region offered an enchanting blend of fertile landscapes and diverse ecosystems. Isabela's heart belonged to the lush fields, rolling hills, and the promise of outdoor adventures that the Californian countryside presented.

Her love for horseback riding and hiking flourished amidst the golden fields and picturesque trails. The allure of the landscape beckoned her to engage in outdoor activities, and she reveled in the vibrant hues of wildflowers that painted the valleys during the spring. The sunsets over the Pacific Ocean, visible on the horizon, cast a mesmerizing glow over the landscape, creating a tapestry of colors that fueled Isabela's passion for the outdoors.

One sunny day, a few weeks after a particularly dusty horseback ride through the trails, Isabela noticed her health taking a subtle and perplexing turn. A low-grade fever, joint discomfort involving her knees, and a mild skin rash seemed to challenge her usual sense of well-being. These were symptoms she hadn't encountered before, and as a diligent high school student with a bright future ahead, she began to wonder what might be causing this discomfort.

Intrigued but not overly alarmed, Isabela decided to pay a visit to her local clinic for guidance. The doctors there examined her carefully, recognizing her discomfort yet failing to pinpoint a clear cause. They suspected a minor allergy due to the skin rash and joint discomfort, prescribing over-the-counter medications with the hope that these would offer her relief.

Weeks passed, and Isabela's symptoms persisted. At this point, it became clear that something more serious might be at play. Her discomfort had not abated, and the prospect of a more in-depth examination seemed inevitable. So, she sought the opinion of a specialist to uncover the underlying cause of her symptoms.

Through further testing, the specialist recognized the need for a more comprehensive evaluation, one that ultimately revealed the correct diagnosis: Valley Fever. The specialist noted that her case was relatively mild and that the fungal infection had not spread beyond her lungs. This discovery marked the start of her treatment for Valley Fever, signaling the beginning of her journey to recovery.

Isabela's treatment for Valley Fever involved a course of oral antifungal medications lasting several months. Regular check-ups monitored her progress, and adjustments were made as needed. Alongside medication, she received guidance on symptom management, rest, and a balanced diet. Adhering to the prescribed regimen, Isabela experienced gradual improvement, with her symptoms subsiding over time. The combination of antifungal therapy and comprehensive care contributed to her successful recovery.

Isabela's experience was a reminder of the often subtle nature of the disease, even in regions where Valley Fever is prevalent. It emphasized the importance of early diagnosis and appropriate treatment. This form of mild Valley Fever was called "desert rheumatism" by doctors in the Central Valley before it was discovered that this was part of the immune response to Coccidioidomycosis.

Scenario

High school students from various counties across California (Fresno, Kern, Los Angeles, Merced, Orange, Riverside, Sacramento, San Diego, San Francisco, and San Luis Obispo) participated in a feasibility study in conjunction with local universities, County Health Departments, and the Centers for Disease Control (CDC) to learn about the presence and prevalence of *Coccidioides* fungi in their local soils. Coccidioidomycosis, commonly known as Valley Fever, had been a concern in the area, prompting the students to engage in a collaborative effort to collect soil samples and contribute to the understanding of the local environmental conditions. The primary goal of the project was to assess the prevalence of *Coccidioides* in different areas within the community, contributing valuable data to the ongoing efforts of local health authorities and research institutions.

Equipped with appropriate safety equipment such as N95 respirator masks and disposable gloves, students collected soil samples in the areas where they live. They utilized sterile scoops and labeled containers for sample collection, ensuring a systematic approach to gathering soil from various locations within each selected site. Each sample, carefully documented with information about the sampling site and characteristics, was sealed securely to prevent contamination and spore release during transport.

After collection, the soil samples were then sent to local universities where the high school students collaborated with microbiology experts to perform tests on the soil samples to detect the presence or absence of the *Coccidioides* fungus. In order to test the soil for the fungus, the soil samples were first processed to extract DNA. This involves breaking open any fungal cells and isolating the genetic material using specialized laboratory techniques. The extracted DNA is then purified to remove any substances or materials that may interfere with the subsequent molecular techniques. The next revolutionary step in this process is known as Polymerase Chain Reaction (PCR) (See Appendix A).

The polymerase chain reaction (PCR) is a molecular biology technique employed in this *Coccidioides* soil testing lab to selectively amplify or make copies of specific fragments of *Coccidioides* DNA. In the PCR reaction, a pair of short DNA sequences known as primers are designed to match unique regions within the *Coccidioides* genome. These primers act as bookmarks at the beginning and end of the DNA fragment to be amplified. The selection of these primers is crucial, as they dictate the specificity of the amplification process. Researchers carefully choose primers that are highly specific to *Coccidioides*, ensuring that only the genetic material of interest is replicated during the reaction. Successful amplification of the *Coccidioides* DNA using these primers would result in a DNA fragment of 223 base pairs (bp).

In addition to these *Coccidioides*-specific primers, researchers also added fungal-specific primers to the PCR reaction mix. These fungal specific primers will amplify DNA from any species of fungus. These primers act as the positive internal control in this experiment, to confirm the successful extraction of fungal DNA as well as successful PCR amplification. Amplification of fungal specific DNA fragments would result in a PCR product of approximately 600 bp. The use of multiple pairs of primers in a single PCR reaction is known as a multiplex PCR

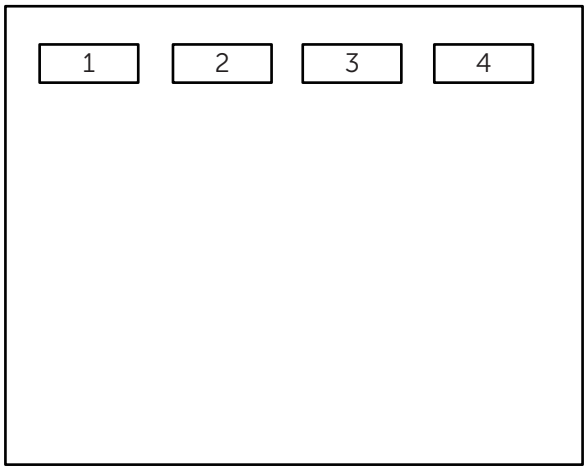
reaction, and allows for the simultaneous amplification of multiple target DNA sequences in a single reaction tube, thus saving time and reagents.

These PCR products were then run on an agarose gel to determine the presence or absence of *Coccidioides* fungus in the soil samples collected by the students in various counties in California.

Analysis Questions

1. Predict what the bands on the agarose gel would look like if the samples were loaded as follows:

   Lane 1 - Positive Control for *Coccidioides*
   Lane 2 - Internal Control for all fungus
   Lane 3 - Soil Sample positive for *Coccidiodes*
   Lane 4 - Soil Sample negative for *Coccidiodes,* positive for other fungus



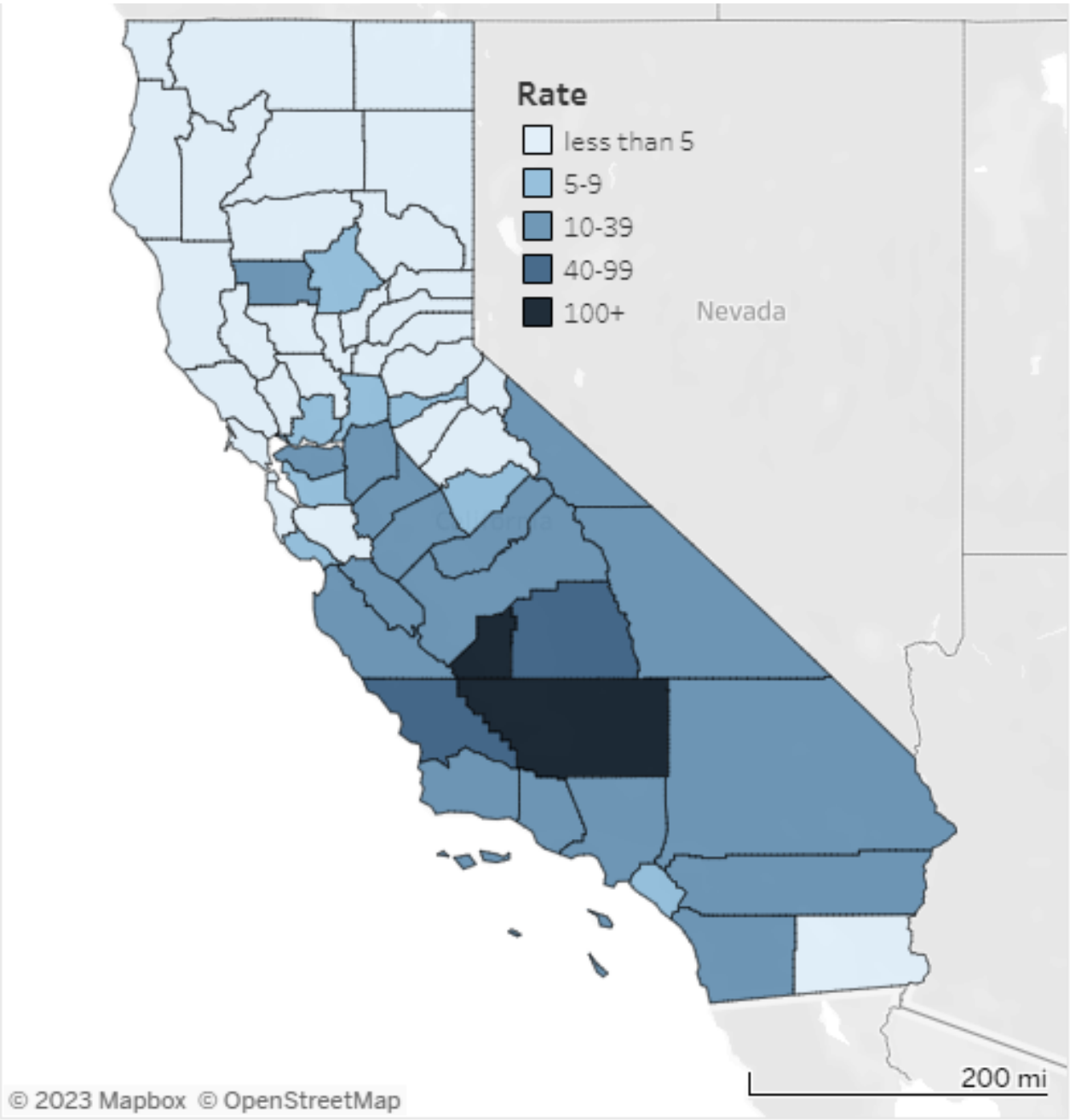
2. What safety precautions need to be taken when collecting soil samples to test for the presence of *Coccidioides* fungus? Why are these safety precautions necessary?

3. Explain the purpose of using a positive internal control in this multiplex PCR reaction.

4. On the map below, locate and put a dot by the names of the ten sampling sites (Fresno, Kern, Los Angeles, Merced, Orange, Riverside, Sacramento, San Diego, San Francisco, and San Luis Obispo).

https://www.counties.org/sites/main/files/file-attachments/california_county_map.pdf?1494366665

5. Compare the counties from question 4 to this map from the California Department of Public Health (CDPH) which shows the incidence of Valley Fever. Predict the incidence of Valley Fever (high, medium, low) for each of the ten sampling locations, then color in the map from question 4 for each county being tested today - red for high, orange for medium, and yellow for low.



https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/ValleyFeverDashboard.aspx

Part I: Electrophoresis

Materials

1 MiniOne® Casting System
1 MiniOne® Electrophoresis System
1 agarose GreenGel™ cup (1%)
1 DNA sample strip
1 MiniOne® Marker
135 mL of running buffer
1 micropipette (2-20µL)
9 pipette tips

Lane #	Sample Name	Volume
1	Positive Control	10 µL
2	Internal Positive Control	10 µL
3	Sample 1	10 µL
4	Sample 2	10 µL
5	Sample 3	10 µL
6	Sample 4	10 µL
7	Sample 5	10 µL
8	Sample 6	10 µL
9	MiniOne® Marker (2000, 1000, 500, 300, 100 bp)	10 µL

How to Cast a Gel

1. Place the MiniOne® Casting Stand on a level surface and place gel trays in the two cavities. For proper tray orientation place the tab edge of the tray on the left side. Insert the comb into the slots at the top of the casting stand with the 9-well side facing down.

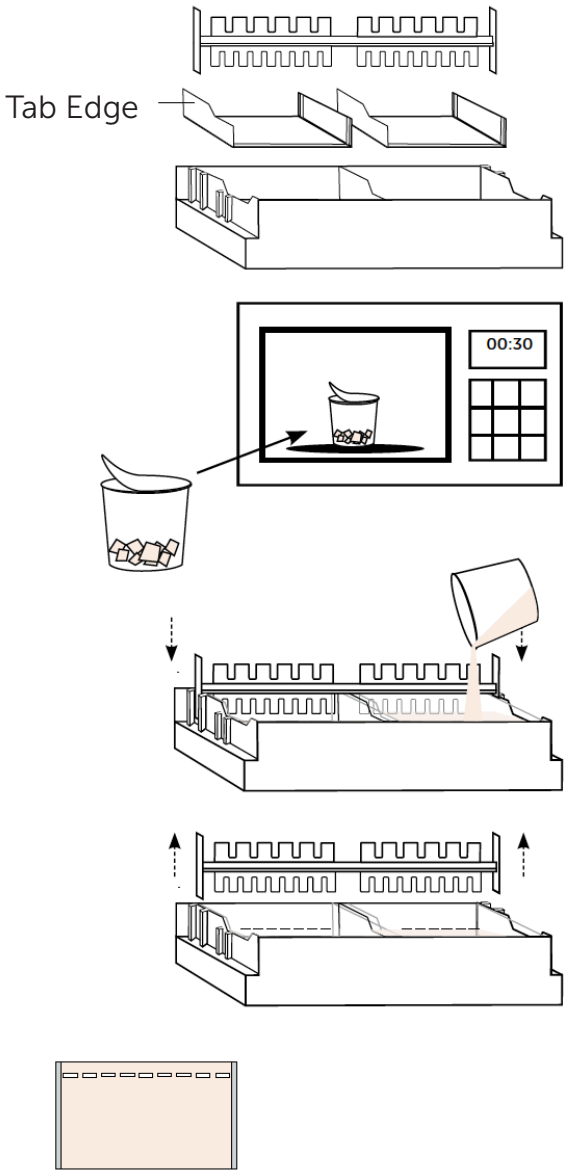
2. **Partially** peel the film off a GreenGel™ cup and microwave for 25-30 seconds. Allow to cool for 15 seconds. DO NOT microwave more than 5 gel cups at a time.

Safety requirement: Adult supervision required if students are handling gel cups!

3. One gel cup is for making one agarose gel! Slowly pour the hot agarose solution into a gel tray. Make sure there are no air bubbles in the agarose solution. Let the agarose gel solidify for 10 minutes or until opaque.

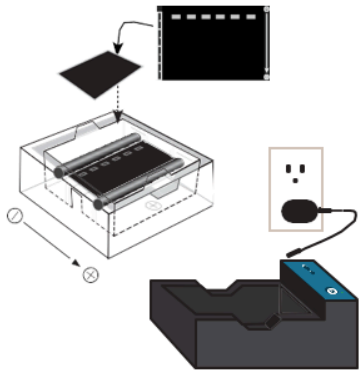
   DO NOT disturb the gel until time is up.

4. Carefully remove comb when gel is ready. Remove gel tray with solidified gel from Casting Stand and wipe off any excess agarose from the bottom of the tray.



How to Load a Gel

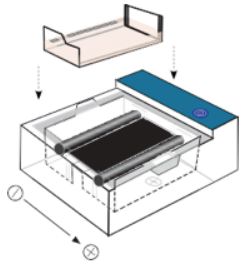
1. Ensure the black viewing platform is in the gel tank. **Make sure the wells are aligned with the marks on the platform on the negative end.**



2. Plug the power supply into the wall and carefully insert the other end into the back of the MiniOne® Carriage.

3. Place the gel tank into the carriage so the carbon electrodes are touching the gold rivets and the tank sits level with the carriage.

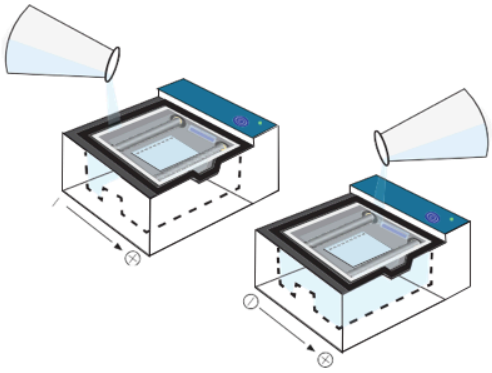
4. Place the gel tray with the gel into the gel tank. The gel tank should not have any buffer in it when putting the gel tray with gel into it.



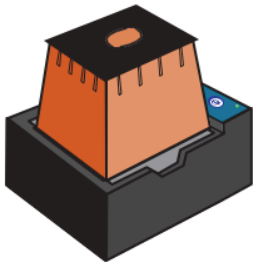
Note: Place gel tank with the gel on the gel tray into carriage **before** pouring buffer in.

5. Turn the low intensity blue LED on by pressing the button on the carriage.

6. Measure 135 mL of TBE running buffer and pour into **one side** of the gel tank. Watch the air push out between the gel tray and viewing platform. Once air has been removed from under the gel tray, pour remaining buffer into the **other side of the gel tank.**

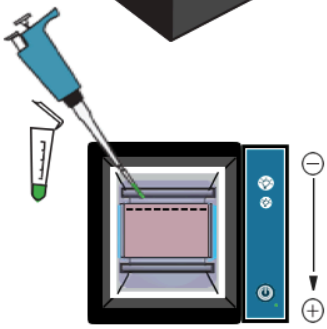


7. Place photo hood on the carriage.



8. Press the power button which should now be a solid green light. If **green light is solid,** turn off the unit and proceed to loading gels.

9. Turn the low intensity blue light on by pressing the button on the carriage to help visualize the wells when loading.



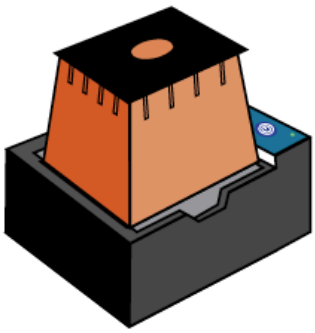
10. Load 10 µL per well by poking your pipette tip through the foil seal. Remember to change pipette tips for each sample. **Load your samples according to the order given in the sample chart.**

Run, Visualize and Capture Image

1. Once the gel is loaded, do not move it. Make sure the power supply is plugged in and place the photo hood on the carriage. Turn on the unit by pressing the button. The green LED next to the button will turn on.

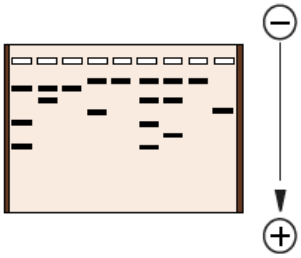
   The green power LED will not turn on if:

   - **The tank is not properly placed inside the carriage. There is no buffer in the tank.**
   - **The buffer is too diluted.**
   - **The photo hood is not on the carriage. There is too little running buffer.**
   - **The power supply is not plugged in. Check by turning on the blue LEDs.**
   - **If the green power LED is blinking, please refer to the troubleshooting steps in the MiniOne Electrophoresis Instruction Manual**



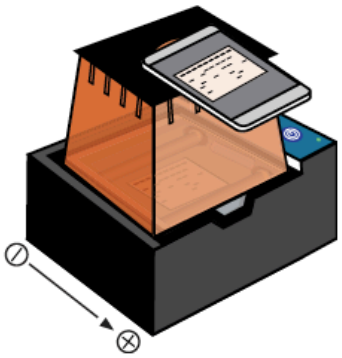
2. Have students periodically check the migration of the bands (~every five minutes).

3. Allow the gel to run **25 minutes** or until DNA separation is sufficient. Keep in mind small DNA samples run faster so it's important to periodically check where your bands are. After your run is complete, turn off the power by pressing the button. Use the low intensity for viewing during the run. Light will weaken the fluorescent DNA signal.



4. Document your results.

   Wipe off the condensation from the inside of the hood with a soft cloth if necessary, then place the hood back on the carriage. **Turn on** the high intensity light. Place your cell phone or camera directly on the photo hood to take a picture of the DNA. **DO NOT** zoom in as this will result in blurry pictures. (The photo hood is already at the optimal focal length for a smart device.



5. Clean up. Follow teacher's instructions on disposal and clean up.

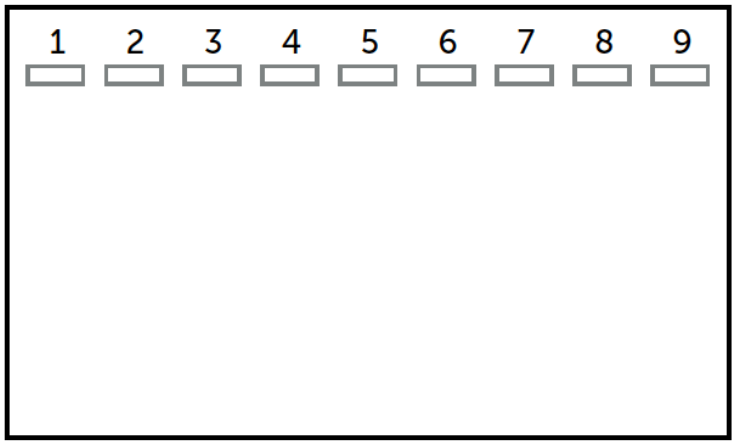
Clean Up

Note: All reagents in this lab can be disposed of as non-hazardous waste.

1. After collecting data and documenting results, remove the photo hood and unplug the power supply from the wall and from the back of the MiniOne® Carriage Remove the clear running tank from the carriage and remove the gel and tray from the running tank.
2. Pour the used running buffer down the drain or into a waste beaker. Throw the gel away AND SAVE THE GEL TRAYS. Rinse the clear plastic running tank, gel tray, comb, and casting system with DI or distilled water. Allow the tanks to fully air dry before storing.
3. Use a paper towel or Kimwipe™ to gently wipe the gold rivets in the carriage (where the electrodes connect) to ensure all moisture is removed. Wipe up any buffer that may have spilled into the black carriage. Follow any additional directions the instructor gives for clean up and storage.

Part II: Results

What does your gel look like? Record images of the gel in the gel below along with the resulting fragment sizes.



Lane 1: _____

Lane 2: _____

Lane 3: _____

Lane 4: _____

Lane 5: _____

Lane 6: _____

Lane 7: _____

Lane 8: _____

Lane 9: _____

Classroom Tally Chart

County	Number of Positive Samples	Incidence of VF (Low, Medium, High)
Fresno		
Kern		
Los Angeles		
Merced		
Orange		
Riverside		
Sacramento		
San Diego		
San Francisco		
San Luis Obispo		

Incidence of Valley Fever:

- Low: 1-2 positives
- Medium: 3-4 positives
- High: 5+ positives

Post Lab Analysis Questions

1. Based on the gel electrophoresis data, rank the incidence of Valley Fever in each of the counties (from highest to lowest).

2. Did the results from the gel electrophoresis match your predictions from the maps on Questions 4 and 5 above? Why or why not?

3. Based on the results of this feasibility study, would you suggest that the local universities, Health Department, and the Centers for Disease Control (CDC) expand this study to the other counties in California, and in other states across the country? Why or why not?

4. How could the findings from this soil testing lab contribute to public health initiatives and community awareness regarding *Coccidioides*?

Optional Culminating activity: As a culmination of our soil testing study for *Coccidioides*, imagine you are tasked with creating a Public Service Announcement (PSA) to raise awareness about the risks of Coccidioidomycosis in your community. This PSA can be in the form of a poster, a video, a website, a social media post or any medium that would reach your target audience.

Be sure to consider the following:

- What key messages would you include in your PSA to effectively communicate the dangers, preventive measures, and the significance of community involvement in minimizing the risk of contracting Valley Fever?
- How would you creatively engage and educate your audience, considering diverse demographics and potential exposure scenarios?

Appendix A - Polymerase Chain Reaction

Polymerase chain reaction (PCR) addresses two major challenges in molecular biology producing usable quantities of DNA for analysis and isolating a specific region of the genome. First, the DNA that scientists want to analyze is often collected in extremely small quantities (for example, a single drop of blood from a crime scene). To have usable quantities of DNA we must make many copies using the original DNA as a template. Second, among the whole genome we must find only the part we want to analyze, whether it is a gene that causes a disease or a sequence that can help identify a species. This is no small matter since the human genome is over 3 billion base pairs (bp) long and we are often interested in regions less than 300 bp long.

The History of PCR

As with many ideas in biotechnology, nature provides us with most of the tools we need. Every time a cell divides it makes two replicates of its entire genome with the help of specialized enzymes. The phenomenon of complementary base pairing should give you a hint as to how a specific region can be targeted. In the late 1970s Frederick Sanger developed a method for copying DNA in vitro that used short pieces of DNA, called primers to initiate replication by a DNA polymerase, similar to the RNA primers in your model of cellular DNA replication. An artificial primer used in the Sanger method has a sequence that allows it to bind at only one location in the target DNA sequence.

Using only one DNA primer, the Sanger method can only produce one copy of the target at a time. In 1983 Kary Mullis proposed a modification to this method where a second primer is used to initiate replication using the first copy as a template. Recall that DNA has an antiparallel structure where one strand runs in the opposite direction relative to the second strand. To use the first copy as a target for replication, the second primer that Mullis added needed to initiate replication in the opposite direction, thus one primer is called the forward primer and the other is called the reverse. With the original sequence and its copy serving as templates, two copies are produced instead of one. Further, these two copies can each be used as templates in a second round of copying, which produces four copies. After each round, or cycle, the number of copies has doubled. Over multiple cycles, billions of copies of the DNA sequence between the two primers are generated. This method is called Polymerase Chain Reaction (PCR) – *polymerase* because of the enzyme that is used to copy DNA and *chain reaction* because the products of one cycle serve as templates for the next round of copying. This rapid and efficient technology for generating usable quantities of DNA won Mullis the Nobel Prize in Chemistry. The first application of PCR was a test for sickle cell anemia.

How PCR Works

Instead of trying to recreate the cell's intricate biochemical machinery in a test tube, scientists rely on heat to control the various steps of the PCR reaction. For DNA to be copied the nucleotide bases must be exposed, which means double stranded DNA must be separated into single strands. Just as heat applied to an ice cube weakens the hydrogen bonds between water molecules and causes a phase transition to liquid water, heat applied to double-stranded DNA weakens hydrogen bonds between bases causing separation of the strands into single-stranded

DNA. As with ice, this is sometimes called melting, but is commonly referred to as denaturation. In a PCR cycle, denaturation is performed at 90-98°C for 5-30 seconds. This temperature is just below the boiling point of water.

Once the template DNA has been separated into single strands, the temperature is lowered to encourage primers to bind to their target sequences. Just as in the water analogy, lower temperature favors the formation of hydrogen bonds between molecules, in this case the primer and the template DNA. This step, called annealing, is typically between 45 and 65°C for 5-30 seconds. The ideal temperature and duration of the annealing step depends on the sequences of the primers being used. It must be low enough that hydrogen bonds are able to form between the primer and specific complementary sequence, but not so low that nonspecific, or random, binding between primer and template occurs.

DNA polymerase binds to the primer-template complex and begins to add nucleotides (dNTPs) onto the 3' end of the primer. This step, called extension, results in a new copy attached to the template as double-stranded DNA. The duration of the extension step depends on the length of the DNA segment being copied and can be anywhere from 5 seconds to 5 minutes.

At this point you may have noticed a problem – DNA polymerase, which the entire PCR process relies on, is a protein enzyme that needs a very specific three-dimensional structure to copy DNA. Before getting to the extension step, the entire PCR mixture, including the polymerase, has already been through the denaturation step where the reaction was heated almost to boiling. Anyone who has cracked an egg into a frying pan will know what high heat does to proteins!

In the early days of PCR, fresh polymerase was added to the reaction tube every cycle to replace the polymerase that had been destroyed by the denaturation step. Having to open the tube and add new enzyme for up to 30 cycles was expensive, inefficient, and increased the chances of contaminating the reaction. The innovation that would remove these obstacles came from an unusual source – Yellowstone National Park.

In the 1970s, scientists had isolated a species of bacteria called *Thermus aquaticus* from a hot spring in Yellowstone. *T. aquaticus* thrives at 75 - 80°C and can survive much higher temperatures. Its enzymes are similarly heat-tolerant. E. coli, the original source for the polymerase used in PCR, thrives at 37.5°C, the same temperature as the gut of mammals.

Since the biochemistry of DNA is similar across the tree of life, polymerase from *T. aquaticus* (called Taq Polymerase) can replace E. coli polymerase in PCR, with the modification that the extension step is performed at 70-75°C.

This innovation led to PCR becoming the ubiquitous, inexpensive, and efficient tool that it is today. Reactions can be set up once, sealed inside a tube, and placed in an automated thermal cycler. The thermal cycler (or PCR machine) is a specialized instrument that accurately and rapidly changes the temperature of the tube between denaturation, annealing, and extension. After 20-40 cycles of these three temperatures, billions of copies of the desired DNA product can be produced. The amplified DNA can be analyzed with gel electrophoresis at the end of the experiment or detected as they are being formed using more advanced equipment.

Appendix B - What is Gel Electrophoresis?

Gel electrophoresis is a technique used in many areas of science to analyze the components of complex chemical mixtures. Mixtures of DNA, RNA, proteins, or dyes can be separated into their individual components based on molecular size and electrical charge using a separation matrix within an electric field.

The gel used in gel electrophoresis is a tangle of polymers forming a three-dimensional matrix with water-filled pores through which molecules migrate. A higher density of polymers creates smaller pores. Like the holes in a sieve or colander, the size of the pores has to be the appropriate size for the molecules being separated. Gels can be made from different substances depending on the application. One of the most commonly used and effective materials is agarose, a polymer extracted from seaweed. Agarose gels are formed (or cast) by pouring molten (melted) agarose into a tray where it solidifies into the desired shape as it cools. A comb is placed while the agarose is molten and then removed after it solidifies to create wells where the samples are loaded.

After the gel solidifies it is placed in an electrically conductive buffer between parallel positive ((+) anode) and negative ((-) cathode) electrodes.

A voltage is applied between the electrodes, creating a uniform electric field within the gel. Molecules in the wells begin to move under the influence of the electric field: positively charged molecules migrate toward the (-) cathode and negatively charged molecules migrate toward the (+) anode.

The speed of a molecule's movement in an electric field is determined by the strength of its electric charge relative to its molecular weight. This is quantified as the charge to mass ratio. Speed of movement within a gel is also influenced by the size of the molecule relative to the pores in the gel. The polymers in the gel are like an obstacle course: smaller molecules maneuver easily through the pores, traveling faster and farther than large, bulky molecules. However, a large molecule can move faster through a gel than a smaller molecule when the strength of its charge relative to its mass is significantly higher. Shape can also affect how a molecule moves through the gel. Long spaghetti-like molecules will move slower than compact molecules, which slip easily through the pores. Molecules of the same size, shape, and charge will move together and form a distinct band. If multiple types or sizes of molecules are present in the sample, they will separate from each other and each will form a distinct band.

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