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# Detection of *Coccidioides* in dust samples collected in Kern County: A community science project

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**Abstract**

The incidence of Valley fever, a disease caused by the fungal pathogen *Coccidioides*, has increased steadily in the Southwestern U.S. over the last decades. To enhance understanding of the pathogen and the disease it causes in the population of Kern County, CA, a known hot spot of this pathogenic fungus, a Citizen Science project was started in 2020. This hands-on inquiry-based project involves students and teachers from California State University Bakersfield and several local high schools. They are using a molecular biological approach to detect the pathogen in dust samples collected by students over the seasons. Being involved in a project that is of interest for the scientific community, students not only develop lab skills needed in scientific careers, but also learn about the importance of being educated on a disease that affects thousands of people each year in California alone.

**Keywords**: Valley fever, community science, dust sampling, diagnostic PCR, emerging disease, *Coccidioides*, fungal pathogen

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## INTRODUCTION

Integrating high-impact research opportunities with tools that are standard in environmental and clinical labs into the science curricula of Advanced Placement (AP) Biology labs at high schools, undergraduate, and graduate institutions is an important practice. This practice can increase students' attendance, curiosity, and participation, as well as enforce critical thinking skills and lab skills. The same holds true for educators who are providing investigative science projects opportunities for their students. This project focuses on Valley fever, also known as coccidioidomycosis. This expanding disease is endemic to the Southwestern U.S., as well as Central and South America, because of climate change and increasing soil disturbance for urban sprawl and renewable energy construction. Currently, no cure or preventative treatment exists for Valley Fever and, despite the risks to a growing number of communities, the disease is not formally discussed in K12 health courses in endemic areas of the pathogen. To help both raise awareness and engage students as co-researchers, we used a community science framework to develop several educational modules. These modules are designed to educate students on i) the ecology of the fungal pathogen *Coccidioides*, ii) symptoms and signs of Valley fever, iii) incidence of the disease, iv) methods to detect the pathogen, as well as v) ways to prevent exposure and possible infection. Co-developed by university and high school faculty, these modules represent the curricular and pedagogical potential of university-school partnerships.

### *Coccidioides* and Valley fever

Parts of the Central Valley of California and the Mojave Desert are endemic to the soil-borne fungal pathogen *Coccidioides,* which is adapted to the Lower Sonoran Lifezone (Merriam 1898). During the dry season, *Coccidioides* survive in the form of barrel-shaped arthroconidia which can easily be disrupted and become airborne when soil is disturbed. Some examples of such disturbances are human activities, digging animals, high winds, or natural disasters such as earthquakes. *Coccidioides* arthroconidia are approximately 2-5 µm in length, which is small enough to remain suspended in the air for hours to days and to be inhaled into the lungs, where an infection may result (Kirkland and Fierer 1996). Valley fever was first documented in California towards the end of the 19th century (Rixford and Gilchrist 1896). The disease is also known in the literature as coccidioidomycosis, desert rheumatism, and San Joaquin Valley fever. Of the two fungal species belonging in the genus *Coccidioides*, *C. immitis* is dominant in California, and *C. posadasii* is dominant in other areas of the Americas (Daubenmire 1938; Centers for Disease Control and Prevention [CDC], 2022). People who spend time outside on dusty days are at risk of inhaling arthroconidia (spores) of the pathogens, which may lead to a lung infection (Smith et al. 1940; Galgiani et al. 2005).

Although about 60% of infections are asymptomatic, and most other patients experience self-limited influenza-like symptoms, rashes, and fatigue (Saubolle et al. 2007), about 1% of patients develop more severe symptoms that can lead to dissemination of the pathogen to skin, bone and meninges of the brain (Ampel 2011). Valley fever has been a concern for some time for several reasons: i) disease incidence is steadily increasing over time, ii) misdiagnosis and delayed diagnosis of the disease is common, iii) misconceptions about the pathogen and disease among the public are leading to an underestimation of the risks associated with contracting the disease, and lastly iv) Valley fever is underreported. Its impact on lost productivity is grossly underappreciated (Thompson et al. 2014).

#### *Community Science*

Community science or citizen science is public engagement in scientific research. Through community science, scientists can expand the scale of data collection, speed the rate of data analysis, and amplify the educational dimensions of their work. For community science participants, they can learn about a community issue, contribute to scientific discovery, ensure their community is represented in the data collection, and bring their unique perspective to data analysis. A Community Science Project was launched in 2020 by faculty at CSUB to increase the education on Valley fever among high school and college students in Kern County. The project started with a workshop for interested teachers at CSUB (Department of Biology), where methods of dust sampling and culture-independent methods to detect the pathogen were

taught. Teachers then developed a lesson plan for Advanced Placement (AP) Biology courses at their schools, and students started collecting dust samples during different seasons at selected locations close to their schools or homes, followed by molecular methods to detect the pathogen. Students were excited about the opportunity to be involved in meaningful research that is relevant to their community.

To further increase education on Valley fever, we suggested presenting this project as a workshop at the ABLE 2023 conference for other teachers outside Kern County, CA. The following flowchart (Figure 1) gives an overview of the basics of this project and how it can be expanded by including side projects. Possible side projects include analyzing soil parameters and working with disease incidence data obtained from the Centers for Disease Control and Prevention (CDC Notifiable Disease Surveillance System (NDSS)). This expansion considers various potential interests of educators and students to expand in more detail into the medical aspects of Valley fever or into the ecology of the pathogen.

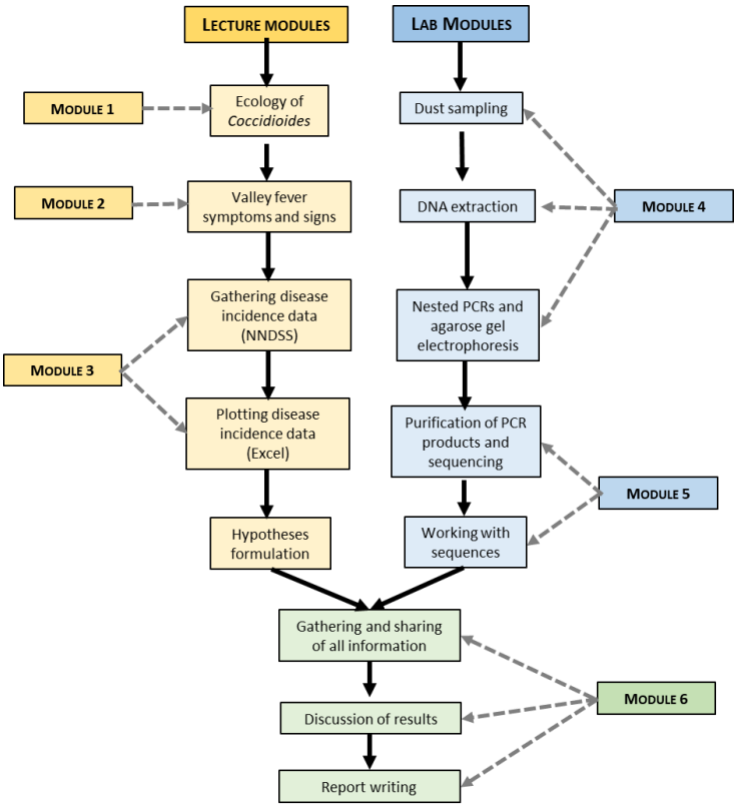


**Figure 1.** Flowchart showing the structure of the exercise separated into lecture and lab modules highlighted in different colors.

ABLE workshop

The workshop on June 28th, 2023, started with an introductory lecture to highlight the ecology of the pathogen, medical aspects of the disease, as well as the disease burden on the community of Kern County, CA. This was followed by an overview of the culture independent methods applied to detect the pathogen which include DNA extraction, a nested PCR approach, and agarose gel electrophoresis. The final part of the project was how to work with DNA sequences obtained from PCR amplicons and how to confirm that your results are reliable, by comparing each sequence to entries in the GenBank nucleotide database. Participants were invited to bring their own dust or soil sample to be investigated.

All methodological steps were explained, and all essential equipment listed. Time for questions was given before the workshop participants started extracting DNA from their own dust or soil sample or from a sample collected in Kern County. There was enough time to finish the extraction and to set up the first PCR of the two PCRs necessary to detect the pathogen using a PCR cycler. The first PCR targets members of the fungal order Onygenales, to which *Coccidioides* belongs. The one-afternoon workshop did not allow for enough time to set up the second PCR using the amplicon from the first PCR with a diagnostic primer pair to detect *Coccidioides*, because the PCR cycling time was about 2.5 hours. However, to expose students to the next steps of the project, prepared PCR products provided by the instructor were used to teach participants on how to perform agarose gel electrophoresis to determine if PCR amplicons of correct size were obtained.

**STUDENT OUTLINE**

## Objectives

Increase awareness of Valley fever in the community of Kern County, CA, to reduce pathogen exposure risk, support early diagnosis of Valley fever or even increase prevention of the disease.

Understand and develop skills to apply molecular techniques, such as diagnostic PCR to detect a pathogen in an environmental sample.

## Introduction

Growing up in Kern County, CA, which is situated in the Central Valley of CA, means being exposed to unhealthy air that lingers most of the year, exposing the population to airborne particulate matter (PM10), which includes microbial spores that can trigger asthma and infectious diseases, such as Valley fever.

In most states of the U.S., including CA, Valley fever is a reportable disease. Public health departments have documented a steady increase in disease incidence since the late 1990's. Reasons for this observation are multifold and include urban sprawl, excessive soil disturbance due to renewable energy construction, off-road biking, the ongoing drought, abandonment of farmland, as well as soil disturbance by rodents and other animals (Wilken et al. 2015; Colson et al. 2017; Gorris et al. 2018). More than 9,000 cases have been reported annually in California since 2019, most of them in the Southern San Joaquin Valley (CDC 2023). Furthermore, the endemic area of the pathogen appears to be expanding due to climate change, resulting in more cases of Valley fever being reported from outside traditional endemic areas in recent years, such as Washington State (Marsden-Haug et al. 2014; Turabelidze et al. 2015; Cat et al. 2017; Pearson et al. 2019). Overall, as of 2017, Valley fever is affecting roughly 24% of the population of the U.S. (~79 million people) who reside in highly endemic areas of the pathogen, such as California, Arizona, western Texas, New Mexico, and Nevada.

Although thousands of people (and animals) are diagnosed with the disease each year, the public is not well informed about the "danger that is lurking in the dust." K12-schools traditionally focus on sexually transmitted diseases in the classroom, which are also on the rise in some areas of the U.S., but endemic diseases such as Valley fever are rarely included in a K12 health related courses.

## Methods and Data Collection

After learning facts about Valley fever, the ecology of the pathogen, and how to detect this fungus in a dust sample using molecular techniques, you will be instructed on how to perform each step of this project correctly, starting with collecting and labeling a sample, and using pipettes and aseptic techniques to extract DNA from a sample without contaminating it. Then you will learn how to perform a PCR reaction, use a PCR cycler, perform an agarose gel electrophoresis, and finally assess if the PCR products that were obtained represent positive results that can be included in the GIS story map.

<u>Part A: Selecting Your Sampling Site and Collect a Dust Sample.</u>

Consult with your teacher about where to collect a dust sample. Mak sure you understand all steps indicated in figure 2 below to ensure that your sample is labeled correctly and not contaminated. Remember to refrigerate, or better, freeze your dust sample prior to DNA extraction (at school or at home). See figure 2 for all steps necessary to collect a dust sample. It is also recommended that you take a photo of your sample site. Furthermore, you might want to record weather data, such as air temperature, and record if it was a windy day when you sampled.

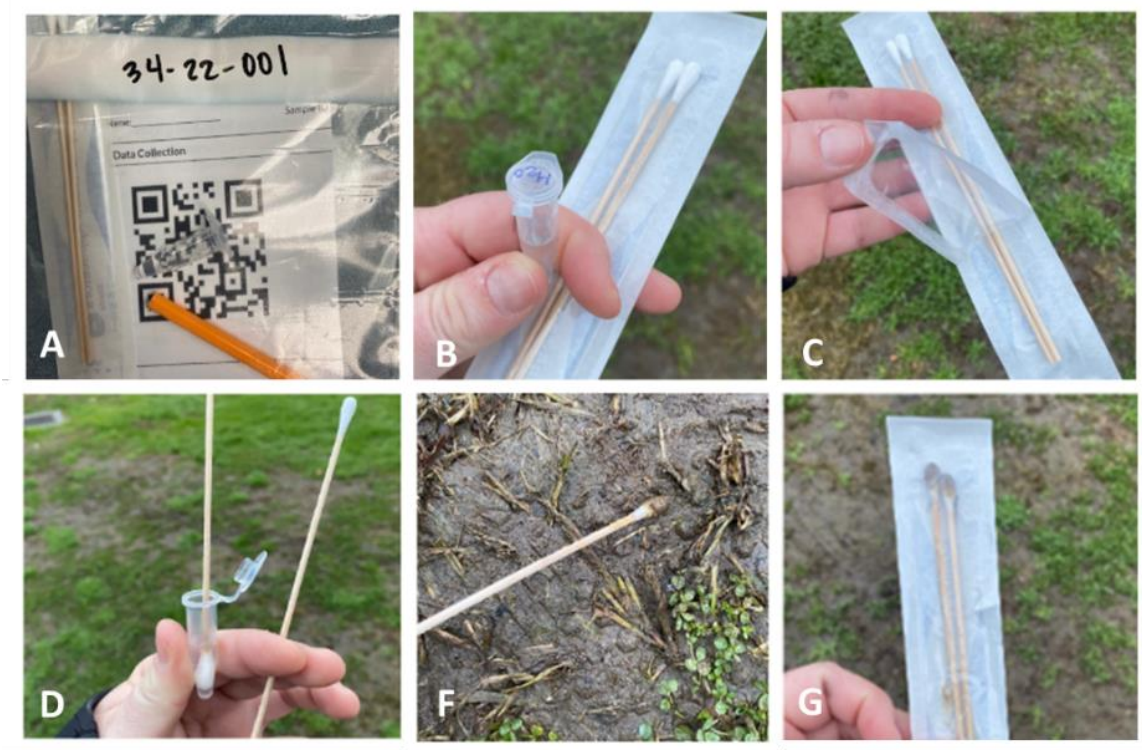


**Figure 2.** Step by step sampling procedure. Your ziplock bag has a 7-digit code. The bag contains 2 swabs, a sample card with a QR code, a 1.5 ml Eppendorf tube with sterile water, and a pencil (**A, B**). Open the sleeve that contains the swabs (**C**). Moisten each swab with sterile water before sampling (**D**). Sample your location with each swab (**F**). Transfer both swabs back into their sleeve, label the sample, and bring it to your school (**G**).

Part B: DNA Extraction, nested PCR, Agarose Gel Electrophoresis, and Sequencing

To help you complete your project, you will be guided through all of the steps including DNA extraction, PCRs and agarose gel electrophoresis. For the DNA extraction, follow the protocol that is provided by the manufacturer of the DNA extraction kit that you use. The Appendix includes a detailed protocol of this process. After completion of the DNA extraction, you will use 10 µl of your DNA extract to perform the first PCR with primer pair NSI1/NLB4 following the recipe in table A in the Appendix. In a similar way, you will perform your second PCR, but with a different primer pair and less DNA (table 1). The PCR cycler conditions are shown in table B in the Appendix.

After each PCR reaction, you will perform a 2% agarose gel electrophoresis to confirm that your amplicons, if any, are of correct size. A 2% agarose gel made with 1x Tris/EDTA/Boric Acid should be performed in 1x TBE buffer with 3 µl of SYBR safe (for a 100 ml gel in a 200 ml Erlenmeyer flask). The SYBR safe is a fluorescent dye that will stain your PCR products and makes them visible when you put your gel on the UV tray when the electrophoresis step is completed. See table C in the Appendix for how to prepare the required agarose gel in the Appendix. If you are using the MiniOne Gel Electrophoresis System, gel material with a fluorescent dye is included and the electrophoresis chamber includes a UV table (https://theminione.com/electrophoresis-system/). To investigate if your PCR was successful, you need to load 4-5 µl of PCR product in individual wells of your agarose gel. A DNA marker (about 10 µl) loaded in the first well will help you to assess if your PCR product is of the anticipated size, which is about 900 bp for the first PCR and about 450 bp for the second PCR. Running your gel at 180V for 25 minutes should be enough time to move your PCR products out of the wells and to spread out the individual fragments in the DNA marker. Take a photo of your gel on the UV tray. Make sure you protect your eyes and skin by doing so. UV light can be very intense and lead to sunburn-like reactions if you are not careful.

Each PCR product of correct size will be treated with ExoSapIt before being sequenced (2 µl of ExoSapIt and

5 µl of PCR product, incubated at 80 °C for 15 min). Freeze all treated positive samples and send out for sequencing (e.g., Laragen, Culver City, CA).

Data Analysis

After sequences are obtained, the next step is to perform a search in the GenBank nucleotide database using the Basic Local Alignment Search Tool (BLAST) to confirm the presence of the pathogen in a dust sample, and to determine accession numbers of closest matches in the database should be recorded with % similarity (https://blast.ncbi.nlm.nih.gov/Blast.cgi ). In addition to the analysis of PCR products obtained with the diagnostic PCR, other data from publicly available databases can be included in this project, such as the proposed NNDSS database. Lastly, you may want to investigate disease incidence data over time or compare case numbers in counties or states to each other, and display results by working with Excel following the instructions provided by your teacher.

## Discussion

At the end of this exercise, you and your laboratory partner will be asked to orally present your results to the other members of your laboratory session. Any difficulties that you encountered will be addressed and discussed in class. Ultimately, the class data will be compiled, and positive and negative PCR results will be displayed on a geographical map that is shared with all students. This can be accomplished at the end of the course together with a critical discussion of the findings. The results of this project can be supplemented by any additional data from public databases, such as disease incidence, and discussed as well. The instructor will provide a detailed guide on what topics to include in this report and how to structure it.

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## EQUIPMENT AND MATERIALS

Equipment and supplies needed to perform this exercise with 24 students is provided in Table 1. This table also includes suggestions of vendors together with estimated costs as of 2023. Keep in mind that K12 schools often obtain a significant discount on supplies. For PCR and gel electrophoresis, we recommend using the equipment and supplies from The MiniOne.

A computer with internet access is required for each pair of students if the students are supposed to obtain disease incidence data from public databases.

It is worth noting that high schools are eligible for special education discounts by vendors such as Fisher Scientific (https://www.fishersci.com/us/en/education-products.html ). Furthermore, high school teachers might want to consider collaborating with a university or community college where equipment for molecular analyses is standard. Certain vendors also offer used laboratory equipment for a fraction of the regular price (e.g. www.labx.com , www.biosurplus.com , http://www.cambridgescientific.com/used-lab-equipment , and even ebay: https://www.ebay.com/b/Lab-Equipment/ ).

To reduce costs, students can work in pairs or in groups of 4. Omitting the purification and sequencing of the PCR products could help in further reducing the costs of this exercise.

**Table 1.** List of equipment and supplies required to perform this exercise with 24 students. The MiniOne for agarose gel electrophoresis includes material for making gels, includes a DNA stain, TBE buffer, and includes a UV tray. The DNA extraction kit provides material and buffers for 50 samples.

| Equipment | Provider/Company | Quantity | Estimated costs ($) |
|---|---|---|---|
| Freezer (-20 C) | The Lab Depot, cat. #ABT-HC-UCFS-0430 | 1 | 1,788 |
| Microcentrifuge (e.g., Accu Spin Micro 17) | Fisher Scientific, cat. # 13-100-675 | 1 | 2,810 |
| Vortexer with adapter (e.g., Vortex Genie) | Fisher Scientific, cat. # 50-728-002 | 1 | 411.43 |
| Vortexter (e.g., Fisherbrand™ Mini Vortex Mixer) | Fisher Scientific | 1 | 317 |
| Micropipette 100-1000 µl | The MiniOne | 6 | 414 |
| Micropipette 20-200 µl | The Mini One | 6 | 414 |
| PCR cycler (e.g., The MiniOne) | The MiniOne, cat. # M1000 | 1 | 959 |
| Agarose Gel Electrophoresis Chamber, (e.g., The MiniOne) | The MiniOne, cat. # M4000 | 6 | 2,004 |
| Sharpie Markers for labeling | store | 24 | 20 |
| **Sample collection and storage** | | | |
| N95 dust mask (only on dusty days) (pack of 20) | Fisher Scientific, cat. # 19-168-175 | 24 | 23.40 |
| Sterile swabs (pack of 200) | Fisher Scientific, cat. # 22-363-163 | 50 | 37 |
| Sterile 1.5 ml Eppendorf tubes (250) | Fisher Scientific, cat. # 01-549-746 | 50 | 98 |
| Sterile water (in 1.5ml Eppendorf tube) | Fisher Scientific, cat. # MT46000CI | 50 | 140 |
| Clean ziplock bags quart | store | 50 | 7 |
| Sample data sheet with QR code | Self-made | 50 | |
| **DNA extraction** | | | |
| DNA extraction kit (e.g., DNEasy Powerlyzer DNA extraction kit - 50 | Qiagen, cat. # 12888-50 | 1 | 396 |

| | | | |
|---|---|---|---|
| Pipette tips for pipettes above (e.g., Aerosol barrier tips 20µl-200µl (pack of 960) | Fisher Scientific, cat. # 02-707-430 | 1 | 152 each size |
| Sterile Nitrile gloves, various sizes (e.g.,Fisherbrand™ Powder Free Nitrile Gloves) (10 packs) | Fisher Scientific, cat. # 19-130-1597C | 1 | 188 |
| Disinfectant (e.g., 10% bleach or 70% ethanol) | Fisher Scientific, cat. # AC615095000 | 6 | 113 |
| Proteinase K | Lamda Biotech, cat. # DB0452-1 | 1 | 62 |
| **Nested PCRs** | | | |
| Sterile PCR tubes (500) | Fisher Scientific, cat. # 05-408-121 or # 14-230-225 | 100 | 54.65 |
| PCR primers (forward and reverse) | Invitrogen, or IDTDNA | 4 | ~100 |
| Green GoTaq Mastermix + sterile water | Fisher Scientific, cat. # PRM7122 | 1 | 94 |
| Positive Control | BEI Resources | 1 | free |
| SYBR Safe | Fisher Scientific, cat. # S33102 | 1 | 87.65 |
| ExoSAP-IT | Affymetrix, cat. # 78200 | 1 | 140 |
| **Agarose Gel Electrophoresis** | | | |
| 0.6% GreenGel™ GelCups TBE | The MiniOne, cat. # M3141TBE | 3 | 90 |
| PCR Marker (e.g., G3161) | Fisher Scientific, cat. # PR-G3161 | 2 | 223 |
| **Sequencing costs** | | | 6/reaction |

Safety in the lab

It is important that students receive instruction about safety measures prior to this exercise. At the beginning of the project, students should be instructed about safety measures during sampling and during work in the laboratory. Any dust sample can potentially contain a pathogenic microorganism. An N95 dust mask is recommended to wear when out sampling on a dusty day. In the lab, students don't need to wear a mask, because the moist swabs were frozen after sampling and will be transferred into DNA extraction tubes before drying up, so the risks of any sampled spores to become airborne is negligent. Regardless, safety procedures should be followed that are essential in all laboratories, meaning students should wear lab coats and nitrile gloves during extraction procedure and PCR preparations and apply aseptic techniques to avoid cross contamination of samples, as well as discarding all waste properly.

**NOTES FOR THE INSTRUCTOR**

Today's biology courses rarely include topics relevant to students' interests. Failing to attract students' attention leads to a lack of understanding of why learning about certain topics benefits their education or might be useful in daily life. Furthermore, concepts of microbiology are rarely included in a high school biology course yet are often an important part of a later undergraduate Biology degree (Huppert et al. 2002, Gasper et al. 2013, McKennye et al. 2016). Student engagement, participation, and learning outcomes can be cultivated by interweaving coursework with applied topics that connect with students, such as Valley fever and its impact on the community. One possible example is to have students explore local community concerns, such as the health risks and strategies on disease prevention. Our exercise proposes using molecular tools that are standard in modern clinical and environmental labs to detect *Coccidioides* in dust samples collected by students, as well as working with disease incidence data and environmental data accessed from public databases. Databases such as the National Notifiable Diseases Surveillance System (NNDSS) are readily accessible. This inquiry-based exercise is student centered and involves them in all steps. In this exercise, students will be encouraged to formulate hypotheses and hypotheses-based predictions, guided by the instructor.

The background on Valley fever, the ecology of the pathogen, and disease incidence data can be used in a lecture of any size. The lab portion, however, was developed for a class of 24 students.

Three modules were developed to cover introductory material on the ecology of *Coccidioides* followed by an introduction on Valley fever and how to gather and work with disease incidence data. A fourth module introduced the theory and application of the methods involved to detect the pathogen, followed by two additional models that focus on how to work with sequence data and how to evaluate results and instructions how to complete this exercise. Understanding of the material can be evaluated by quizzes, during or at the end of a presentation or lecture (Table 4). We suggest that students work in pairs or small groups (table of 4) in the laboratory.

Additional information for module content:

**Modules 1 and 2:** Students acquire information from peer-reviewed literature and from the website of the Valley fever Institute in Bakersfield, CA (http://valleyfeverinstitute.com/), the Center of Disease Control and Prevention (CDC) (https://www.cdc.gov/fungal/diseases/coccidioidomycosis/index.html), the Valley Fever Center for Excellence (https://vfce.arizona.edu), the book 'Valley Fever Epidemic' by David and Sharon Filip, ISBN-13: 978-0979869259 (http://www.Valleyfeverepidemic.com/).

**Table 4.** Suggested lesson plan, indicating learning content, student activity and time required for the different lecture and lab modules for this exercise.

| Lecture | Phase of Teaching | Learning Content | Students Activity | Time (minutes) |
|---|---|---|---|---|
| **Module1:** Ecology of the Pathogen | Introduction to *Coccidioides* | Introduction to the ecology of *Coccidioides* | Discussion and Quiz | 30 minutes |
| **Module 2:** Valley fever | Introduction to Valley fever, Air pollution and Human Health | Background of environmental and human health concerns surrounding Valley Fever, Signs and symptoms of Valley fever, Presentation of a case study | Interviewing a patient who has had Valley fever or investigation of case study (literature) Discussion and Quiz | 60 minutes |
| | C Science Project | Education on Valley fever to aid in disease prevention and early diagnosis | Discussion and Quiz | 30 minutes |
| **Module 3**: Valley fever incidence | NNDSS database | Gathering incidence data | Graph data (Excel) and Homework (complete graph) Formulate hypotheses and predictions | 45 minutes |
| **Lab** | | | | |
| **Module 4:** Sampling and Analysis | **A.** Dust Sampling | Sampling without contamination, Correct labeling of sample | Visual demonstration outdoors, Homework (collection of samples) | 30 minutes |
| | **B.** DNA Extraction | Understand extraction procedure, Learn how to work with pipettes and small sample volumes | DNA extraction from dust samples, label, and freeze | 4 class periods at 55 min each |

| | | | | |
|---|---|---|---|---|
| | **C.** Nested PCR/ Electrophoresis | Theory of PCR and agarose gel electrophoresis | Perform PCR and agarose gel electrophoresis, documentation of results | 120 minutes |
| **Module 5:** Working with sequences | GenBank/BLAST nucleotide search | Introduction to GenBank and its application | Blast search of sequences in nucleotide database, documentation of results, combine class results | 45 minutes |
| **Module 6:** Finish up | Discussion of results | Compiling and sharing of all data, Report Writing Guide | Final report writing and submission | 45 minutes |

**Module 5:** Students learn how to use the GenBank nucleotide database at the National Center for Bioinformatics and Technology (NCBI) to analyze their sequences (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome ).

After covering the background information, students learn and apply laboratory skills needed for DNA extraction processes, PCR, gel electrophoresis, and working with sequences over a period of at least 4 labs, depending on the progress of the class. A last module is suggested to bring all results together, discuss the outcome of this exercise, refer to any hypotheses that were formulated, or any difficulties students encountered when working in the lab. Additional material and original references should be provided to all students at the beginning of this exercise. Figure 3 shows examples of results of the nested PCR.

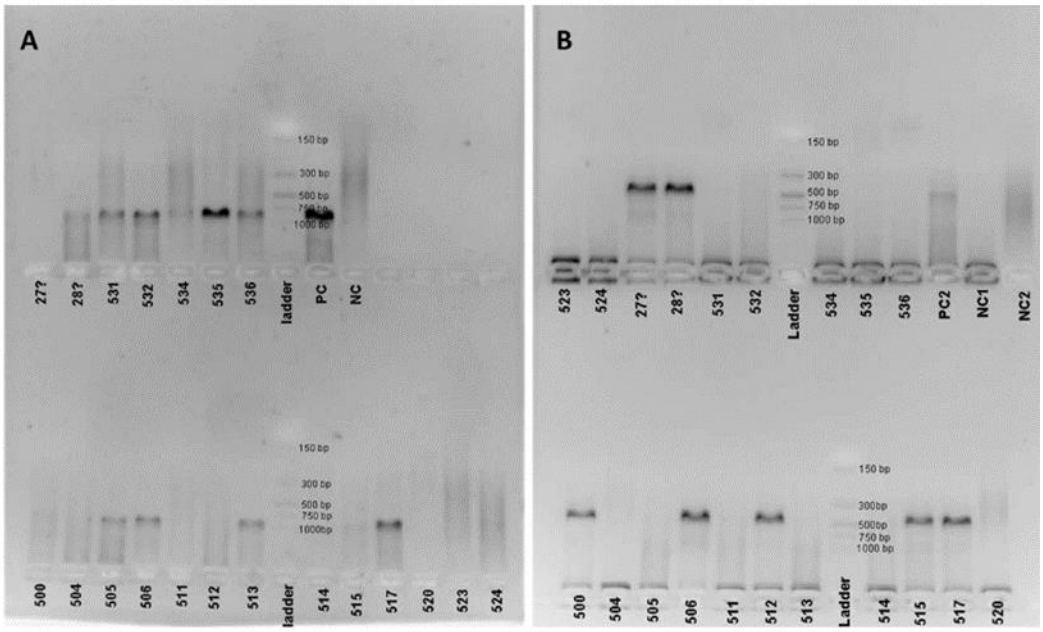


**Figure 3. A:** Results of nested PCRs obtained with primer pair NSI1/NLB4, indicating the presence of members of the Onygenales in many soil samples (~900 bp). **B:** *C. posadasii* was detected in several dust samples in a nested PCR with primer pairs EC3/EC100 (~500 bp), collected in east Bakersfield (Oscar's samples) (PC=positive control, DNA from C. immitis, NC=negative control).

Student Assessment

Students are assessed regarding their understanding of the topic via two conceptual quizzes early on in this exercise. The first quiz includes questions about the life lifecycle of *Coccidioides*, symptoms and signs of Valley fever, endemic areas, at-risk population, factors that cause increase in disease incidence, and the soil environment as a habitat for the pathogen. Students take this assessment after the instructor gives two lectures on the topic and students have assessed disease incidence data from the NNDSS website. The second quiz assesses comprehension of methods, primarily regarding performing DNA extractions and PCR procedures, gel electrophoresis and sequencing. Assessment includes questions about the theories and applications behind these methods. These quizzes reveal whether students are ready for the interpretation of results of this project. An instructor facilitated discussion focusing on student experimental results, with reflection on the earlier formulated hypotheses, is also used to further assess student learning outcome. Methodological issues that are encountered during DNA extractions and PCR are explored during the discussion assessment. Finally, students write individual lab reports with analyses of class results using the format and structure of a research manuscript following a writing guide provided by the instructor (Introduction, Material and Methods, Results, Discussion, References).

Exercise Variations

Teachers in states where incidence of Valley fever is low or absent, can adopt the general structure of this exercise with another opportunistic soil-borne fungal pathogen in the focus, for example *Histoplasma capsulatum* (most common fungal pathogen in the U.S.), *Cryptococcus* spp. *or Blastomyces dermatitidis*. Diagnostic primer pairs for these pathogens can be found in peer-reviewed literature using Google scholar, e.g., McCarthy & Walsh 2016 and references within. Soil borne bacterial pathogens such as *Clostridium tetanii*, *Clostridium botulinum*, or *Bacillus anthracis*, can also be detected via diagnostic PCRs and could be the target of a similar exercise by using the open-internet-accessible Insignia program (http://insignia.cbcb.umd.edu ) to produce DNA signatures for these species (see Phillippy et al. 2009; Nagamine et al. 2015) that can be used in PCRs. Teachers who are concerned about safety of their students can focus on non-human-pathogenic fungi (e.g., some *Penicillium* or *Fusarium* spp.) or plant pathogens such as *Phytophtora* spp., by focusing on developing a soil sampling plan and performing DNA extractions and PCR, excluding working with the NNDSS database (CDC 2023).

It is worth noting that depending on the class period length, it can be particularly challenging to complete some of the lengthy labs and find appropriate stopping points, unless block periods or dedicated lab sections are available at the high school level. This time constraint posed a significant hurdle in ensuring that students could fully engage with and comprehend the intricacies of the biotechnology exercises.

Furthermore, training students to proficiently use the biotechnology equipment and maintain the integrity of the samples proved to be another challenge. Given the complexity of the procedures involved, conducting the lab as a whole group the first time became integral to guaranteeing the correct processing of each sample. This collaborative approach allowed for collective problem-solving and enhanced understanding of the experimental steps.

Lastly, we recommend that the guide to write the lab report includes guidance on report structure, as well detailed information about what is expected in each section (Introduction, Material and Methods, Results and Discussion, References). This proactive approach will motivate students to master basic concepts, apply their knowledge to an applied project, and allow them to practice and improve writing skills.

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## APPENDIX A

**DNA extraction Procedure**

Note for instructors:

Note: Teacher should perform these steps before students arrive to facilitate the extraction process:

1. Set your incubator or water bath to 56 °C.
2. Cut off the "fluffy tips" of your sample swab and transfer it into the powerbead tube (provided by the DNA extraction kit). Then, label with 3-digit ID immediately.
3. Add 750 µl of the powerbead solution (provided by the DNA extraction kit) to the powerbead tube.
4. Add 10 µl of the prepared Proteinase K stock solution to the same tube.
5. Incubate at 56 °C for 30 minutes.

Furthermore, if you don't have a freezer in the lab, put a Styrofoam box with ice on each table. We suggest that students work individually or in pairs. Copies of the DNA extraction process should be provided to each student.

A *Coccidioides* strain that is non-pathogenic and suitable for a BioSafety 2 lab can be purchased for free from BEI Resources with instructions how to grow it. DNA can be extracted from a culture using the same DNA extraction kit that is being used to process the dust samples. See https://www.beiresources.org/Catalog/Fungi/NR-4548.aspx for more information.

Notes for students:

The detailed protocol for the DNA extraction process provided by the manufacturer of the DNA extraction kit. Here are a few other things to consider:

1. Clean your table with a disinfectant.
2. Collect all material that you need, such as pipettes, tips, tubes and racks, extraction protocol and kit, a sharpie marker for labelling.
3. Make sure all buffers and spin columns are present in the DNA extraction kit (somebody else might have used it before).
4. Wear nitrile gloves and your lab coat.
5. Use a fresh pipette tip whenever it came in contact with a sample to not cross contaminate samples or buffers!
6. Mix your sample whenever you added a new buffer.
7. Discard all waste properly.
8. Make sure you balance the microcentrifuge properly and you use the correct speed and time.
9. A supernatant refers to the liquid phase of your sample, the pellet to the solid part that forms after centrifugation.

**Table A.** Recipe for nested PCR to detect *Coccidioides* in dust samples.

| **First PCR (members of the *Onygenales*)** | | **Second PCR (*Coccidioides* specific)** | |
|---|---|---|---|
| **Reagent** | **Amount (µl)** | **Reagent** | **Amount (µl)** |
| Green GoTaq Mastermix | 12.5 | Green GoTaq Mastermix | 12.5 |
| Forward primer NSI1 | 1.5 | Forward primer EC3 | 1.5 |
| Reverse primer NLB4 | 1.5 | Reverse primer EC100 | 1.5 |
| Sterile water | 0.00 | Sterile water | 7.5 |
| Extracted DNA | 10.0 | Extracted DNA | 2 |
| **Overall volume of PCR reaction** | **25.5** | | **25.5** |

**Table B.** PCR cycling conditions for nested PCR based on the original references (Vargas-Gastelum et al. 20012; Johnson et al. 2014) but modified slightly.

| | **First PCR (members of the *Onygenales*)** | | **Second PCR (*Coccidioides* specific)** | |
|---|---|---|---|---|
| | **Degrees (F)** | **time** | **Degrees (F)** | **time** |
| Initial Denaturation | 94 | 4 | 94 | 4 |
| Denaturation | 94 | 30” | 94 | 45” |
| Annealing | 60 | 40” | 56 | 45” |
| Extension | 72 | 60” | 72 | 45” |
| Final Extension | 72 | 5’ | 72 | 10’ |
| Cooling | 4 | Forever | 4 | forever |

**Table C**. Recipe for a 2% agarose gel (100ml).

| **Reagent** | **Amount** |
|---|---|
| Low melting agarose | 2 g |
| 1x TBE | 100 ml |
| SYBR Safe | 2-3 µl |
| Microwave until clear and pour in gel casket, add spacer and let solidify in the dark (e.g., 20 min in the fridge) | |

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