**Outline of Lectures and Laboratories**

**Lecture 1 outline:**

* Introduction to valley fever (see sources indicated in main text)
* Introduction to the USDA WSS database

**Assessment of student learning outcome:**

Quiz 1 (suggested questions) (\*critical thinking question)

1. Name the pathogen(s) that cause(s) valley fever.

*Answer:* *Coccidioides immitis* and *C. posadasii*

1. Why is valley fever an endemic disease? Briefly explain.

*Answer:* The area where it is established is geographically limited

1. Why is valley fever called an “orphan disease”? Briefly explain.

*Answer:* It is neglected regarding funding for research and education because the number of cases in the past were not alarming.

1. How can a physician diagnose valley fever? Name the most common symptoms a patient might experience.

*Answer:* Fever, rash, substantial weight loss, dry non-productive cough leading to pneumonia.

1. Name an unusual symptom that makes it difficult for the physician to correctly diagnose valley fever.

*Answer:* Arthritis (valley fever is also called “desert rheumatism”)

6\*. Why is valley fever increasing in recent years in endemic areas?

*Answer:* Valley fever is increasing because of increased soil disturbance followed by increased dust emissions in endemic areas of the pathogen (housing, renewable energy, roads, dirt biking etc.)

7\*. Could climate change have an influence on the spread of *Coccidioides*? Defend your answer.

*Answer:* Yes, a drier climate leads to an increase in dust emissions. Also, the endemic area of the pathogen might expand (*C. immitis* was recently isolated from eastern Washington State).

1. Name 2 physical and 2 chemical soil parameters that can be obtained using the USDA WSS.

*Answer:* Some physical parameters are water content, percentage of clay, sand and silt, percent organic matter. Chemical parameters are pH, electrical conductivity, calcium carbonate content etc.

9.\* The pH of a soil sample that you measured in the laboratory was different from the value it is supposed to be based on the USDA WSS database. How can you explain this discrepancy?

*Answer:* The values in the USDA WSS database are just averages and might not represent exactly individual soil samples.

10.\* Briefly describe how you would develop a soil sampling plan using the USDA WSS database.

*Answer:* An ideal soil sampling plan should optimally cover all different soil types in the sampling area. The USDA WSS indicates different soil types in a sampling area and sampling can occur along a transect that covers all of them. Averaged soil parameters can also be obtained from the USDA WSS database and can be incorporated into the sampling plan. Multiple samples should be collected from each individual soil type.

* Homework: proposing a meaningful soil sampling plan will be graded.
* Find 2 recent peer-reviewed papers that focus on valley fever and summarize their findings (graded)

**Lecture 2 outline:**

* Introduction to DNA extraction (covering the content of the manufacturer’s protocol)
* Introduction to the Polymerase Chain Reaction (PCR) and gel electrophoresis
* Introduction to purification of PCR products and sequencing

**Assessment of student learning outcome:**

Quiz 2 (suggested questions)

1. What is the purpose of the bead beating step in the DNA extraction procedure?

*Answer:* Bead beating detaches microbes from soil particles and breaks the cells, so that DNA can be extracted.

1. What is the purpose of the spin filter during the DNA extraction process?

*Answer:* The spin filter will collect the extracted DNA.

1. What is the enzyme needed to perform PCRs?

*Answer:* DNA polymerase

4.\* Why is the DNA polymerase from *Thermus aquaticus* used in the PCR and not the one from *E. coli*? Briefly explain.

*Answer: T. aquaticus* is a hyperthermophilic bacterial species that tolerates the high denaturation conditions of 94-95 °C during the first step of each PCR cycle.

5. Name the 3 steps of the PCR reaction.

*Answer:* Denaturation, Annealing, Extension

6.\* Why should a PCR marker be run side by side to your PCR products? Briefly explain.

*Answer:* The PCR marker or DNA ladder allows to assess the correct size of the PCR amplicons.

7.\* Why are primers used that target the ribosomal gene and not any other gene? Briefly explain.

*Answer:* Currently the ribosomal database is the largest. Theoretically any gene can be used in a diagnostic PCR when species specific hypervariable areas are present, and sequences are available in a publicly accessible database, such as GenBank.

8.\* Why is sequencing of the PCR amplicons recommended? Briefly explain.

*Answer:* Occasionally false positive amplicons arise from annealing errors. Sequencing will reveal those.

* Homework: reflecting upon the molecular tools learned to be prepared for the laboratory part. Identify questions to be asked before the begin of the laboratory part (not graded)

**Laboratory 1 outline**

* Introduction to the NNDSS Morbidity Tables
  + Explain difference of incidence and prevalence
* Accumulation of incidence data on valley fever
* Generating graphs with the program Excel that shows increase incidence over time or compares incidence in different counties
* Homework: Completion of soil sampling plan (if not completed earlier) (graded), and completion of disease incidence graph (graded)

**Laboratory 2 outline**

* Review of introductory material,
* performing DNA extraction and agarose gel electrophoresis
* Homework: none

**Laboratory 3 outline**

* Review of introductory material
* Performing PCR and agarose gel electrophoresis
* Homework: none

**Lecture 3 outline**

* Acquiring scientific writing skills (e.g. <https://www.csub.edu/biology/_files/How%20to%20Write_14.pdf>)
* Homework: Laboratory report (graded) including
  + - Introduction
      * Background on valley fever
      * Hypothesis
    - Material and Methods
      * Soil sampling plan
    - Results
      * PCR
      * Valley fever incidence graph
    - Discussion and Conclusion
    - References