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/solution-manual-laboratory-applications-in-microbiology-a-case-study-approach-2e-chess

Exercise 1
Safety Considerations in the Microbiology Lab

Laboratory Objectives: This exercise serves as an introduction to the safety and organizational concerns in the microbiology laboratory.

Overview: Two cases of laboratory acquired infections and one incident of fatal burns suffered in the lab are introduced in the Case Synopses. The first case involves a laboratory worker who was infected with an *Escherichia coli* O157:H7 isolate. Genetic analysis showed it to be identical to an isolate the worker has processed three days earlier. The second case involves a graduate student in immunology suffering from an ocular infection with vaccinia virus, the same virus she worked with in the laboratory. The final case centers on a research assistant at UCLA who suffered fatal burns during a laboratory accident.

Resolution of the cases reveals that lapses in safety procedure were most likely responsible for the infections seen in the cases. In the burn case, a combination of inadequate training, along with lack of proper safety equipment allowed a minor situation to become far worse than it should have been.

Time required: 30 minutes

Instructor preparation

No laboratory manipulations are performed in this lab. It is advisable for the instructor to spend a few minutes comparing the exercise with the physical setup of the actual laboratory, noting any differences in procedures or equipment so that these may be pointed out to students.

Answers to Questions:

Pre-lab

1. *Marburg virus* BSL-4
   M. tuberculosis BSL-3
   B. subtilis BSL-1
   C. tetani BSL-2

2. Negative airflow prevents contamination of the surrounding environment in the event of a laboratory accident.

   Gloves, safety glasses and a lab coat protect the user against spills, splashes and related accidents, either their own or those of someone else in the lab.

   Vaccination of laboratory workers protects the worker against infection in the event that other protective measures fail.

Foot petal activation of sinks reduces the chance of sink handles acting as common vehicles, passing infectious agents from person to person.

Prohibitions on eating and drinking in the lab keep potentially contaminated food and fingers away from the mouth.

3. Petri dishes should be taped closed and placed in the biohazard bag. Marks need not be removed as plastic dishes are not reused.

   A glass culture tube should be cleansed of marks or tape on the outside only, with no attempt made at decontamination. The tube should be placed in a rack or container for autoclaving.

   A hypodermic needle should be placed carefully in a hard-sided biohazard container. Recapping of needles should be avoided.

   A spill containing broken glass and a bacterial culture should be reported to the instructor prior to cleaning. Cover the spill with paper towels and saturate with disinfectant for twenty minutes. Finish by carefully cleaning the spill, disposing of the broken glass in the sharps container and paper towels in the biohazardous trash.

4. Answers will vary.

Review Questions

1. *E. coli* infection most probably began when gloves were not removed prior to using items within the lab, such as the telephone and computer keyboard, leading to contamination. Using the same items later, without gloves, contaminated the hands which led to ingestion when fingers touched the mouth. Gloves should always be removed prior to using laboratory equipment that may be used later without gloves.

   The key in the burn "accident" is that it may not have been preventable. Accidents will after all occur, even to the most skilled hands. However, the outcome could have been far less tragic had the researcher been wearing a lab coat over her highly flammable sweater and working behind a shield. It is also unclear that she knew where in the lab fire extinguishers and laboratory showers were located. All of this points toward inadequate training and a lack of understanding of the true dangers inherent in any laboratory.

   Several laboratory practices could have led to the initial vaccinia virus infection; protective eyewear was not always worn, pipettes containing live viruses were not disinfected prior to being removed from biological safety cabinets, small amounts of live virus were occasionally manipulated outside of the cabinets and no one in the lab had been vaccinated against vaccinia virus. The student was also unable

to remember if she always wore gloves when working in the lab. Correcting these deficiencies, as well as never touching the eyes, nose or mouth in the laboratory, would have lessened the possibility of contamination.

Exercise 2
Microscopy and Measurement of Microscopic Specimens

Laboratory Objectives: This exercise introduces the proper care and use of the brightfield microscope, the underlying theory of microscopy and the procedure for using ocular and stage micrometers to measure specimens. Darkfield and phase contrast microscopes are briefly described.

Overview: The Case Synopses contain excerpts of letters from Anton van Leewenhoek to the Royal Society of London for the Improvement of Natural Knowledge, describing the microscopic organisms he was able to see when collected rainwater and dental plaque were observed microscopically. The Resolution of the case identifies these organisms based on their descriptions as well as further examination of his specimens with modern instruments.

Time required: 45 minutes.

Instructor preparation

Each student should have access to:
Lens tissue*
Prepared slides of:

- Bacteria*
- *Vorticella* or *Spirogyra**
- Paramecium*

Stage and ocular micrometers (If microscopic measurements will be attempted). It is generally easiest to have a few microscopes set up in the front of the lab that have already been equipped with stage and ocular micrometers. This limits the swapping of oculars on the scopes which is a common cause of dirt entering the microscope.
*www.wardsci.com

Preparation for this lab is minimal. Learning to correctly work with a light microscope can be a frustrating experience for some students, especially those who feel that simply by turning knobs and sliding levers they will eventually happen upon an adequate image. It is important that students develop a hierarchy of procedures and manipulations, even an informal one, which allows them to craft an adequate image of their specimen. Once students are used to such a hierarchy, they will be able to properly adjust light sources, clean lenses and otherwise optimize their microscopes in the future.

Answers to Questions:

Pre-lab

1. Definitions
   Magnification: The creation of a larger-than-life image.

   Resolution: The clarity of an image produced by a set of lenses.

Contrast: The degree of difference between the lightest and darkest parts of an image.

Ocular micrometer: A circular glass disc inscribed with a series of regularly spaced marking. The ocular micrometer can be placed in one ocular of a microscope and used, after being calibrated, to measure specimens.

Objective: The microscope lens nearest your eye.

2. The condenser should be adjusted so that the upper surface of the lens is just below the level of the stage. The diaphragm should be adjusted so that it is almost completely open.

3. Both ocular and objective lenses should be cleaned using lens tissue and, if needed, a solvent approved for use in the laboratory such as ethanol or xylene. The condenser lens may be cleaned in the same way but its cleanliness is not as important to the formation of a clear image.

4.

Total Magnification	Ocular Magnification	Objective Magnification
100x		
450x		
1000x		75x
	5x	

Resolution	Wavelength	Numerical Aperature
610 nm		
1068 nm		
244 nm		

Review Questions

1. Both parcentric and parfocal refer to a set of objective lenses that have been matched to one another. Parcentric means that when a specimen is in the center of the microscopic field, it will remain centered when different objective lenses are moved into the light path. Parfocal refers to the fact that when a specimen is in focus it will remain in focus as the objective lenses are changed

2. A 100x objective would increase magnification but, because resolution is limited to about two-tenths of a micrometer, regardless of magnification, the image produced would lack clarity.

3. Total magnification is 675x.

4.

Wavelength of light	Numerical aperture	Resolution (μm)	Distance between points	Resolvable
		381		yes
		339		no
		254		yes
		458		yes
		305		no

5.

a. 5.88 μm /unit
b. 2.35 μm /unit
c. 0.52 μm /unit
d. 0.24 μm /unit