**SECTION C**

**Answers to Questions on Laboratory Reports**

**CV refers to the Complete Version of Benson’s Laboratory Manual, 13/e**

**SV refers to the Short Version of Benson’s Laboratory Manual, 13/e**

**Exercise 1**

**Brightfield Microscopy**

A. Short Answer Questions

1. One hand should be under the base of the microscope to support its weight, and one hand should be on the arm for balance.

2. The limit of resolution of the unaided human eye is 0.2 mm. For the typical light microscope, the limit is 0.2 µm.

3. a. The condenser height and diaphragm can be adjusted.

b. Illumination of the specimen is increased when the condenser is raised and the diaphragm is opened.

4. Unlike the voltage control, condenser adjustments will increase illumination without affecting the bulb life.

5. The maximum resolution with the oil immersion lens is achieved by using a layer of oil, using a blue filter over the light source, raising the condenser to its highest point, and opening the condenser diaphragm.

6. The oil immersion lens has the smallest working distance and one runs the risk of striking the slide with the lens when trying to achieve focus. Starting with the low power lens, which has a larger working distance, and progressing up to the oil immersion lens is advised.

7. Oil is used with the oil immersion lens because the small working distance does not allow enough light to enter the lens. The oil, which has the same refractive index as glass, directs more light into the lens (limits the loss of light due to refraction).

8. As the power of the objective lens increases, the working distance decreases.

B. Matching Questions

1. Oil immersion

2. High-dry

3. Low power

4. Oil immersion

5. Low power

6. Condenser

7. Ocular

8. Ocular

9. Condenser

C. True-False Questions

1. False

2. True

3. False

4. False

5. True

D. Multiple Choice Questions

1. e

2. e

3. c

4. d

5. a

6. d

**Exercise 2**

**Darkfield Microscopy**

A. Short Answer Questions

1. Darkfield microscopy is preferred for live unstained specimens or thin cells like spirochetes that are difficult to resolve by staining and brightfield microscopy.

2. Reflection of oblique rays off of objects passes through the lens system.

3. A cardioid condenser allows more light to pass through the lens system and allows use of more powerful objectives.

4. A simple star diaphragm can be made by cutting various sizes of round disks of opaque paper and cementing them to transparent celluloid disks that fit in the slot.

**Exercise 3**

**Phase-Contrast Microscopy**

A. Short Answer Questions

1. Staining kills live cells and does not allow observation of movement.

2. Direct rays are produced when light passes straight through a transparent medium without changing amplitude or phase. Diffracted rays are produced when light is bent during retardation by the medium due to density differences and are phase-shifted ¼ wavelength.

3. Coincidence results when direct and diffracted waves are brought into phase with one another, where the amplitude is the sum of the two waves, and creates a brighter image. Interference occurs when two waves of equal amplitude are in reverse phase and cancel each other to produce a dark image. Coincidence and interference create greater contrast in specimens being viewed.

4. Bright-phase microscopy produces a brighter image (amplitude summation) with a dark background while dark-phase microscopy produces a dark image (amplitude interference) and a lighter background.

5. Centering telescope or Optovar.

B. Multiple Choice Questions

1. d

2. a

3. a

4. d

**Exercise 4 (CV only)**

**Fluorescence Microscopy**

A. Short Answer Questions

1. Both are types of photoluminescence. Fluorescence occurs when the excited molecule returns to ground state in less than 10–4 seconds. Phosphorescence occurs when an excited molecule returns to ground state in more than 10–4 seconds and has a longer half-life.

2. The electrons of the molecule become excited and are promoted to a high energy (singlet) state.

3. Quenching occurs when fluorescence of a molecule diminishes during prolonged exposure to UV light.

4. Direct exposure to light from mercury vapor arc lamps can damage eyes and the pressurized gas creates the potential to explode.

5. The warm-up period is typically a minimum of 30 minutes where the illumination of the lamp increases to its optimum.

6. A darkfield condenser provides the best contrast and it deflects the majority of UV rays, which protects the viewer’s eyes.

7. Low-fluorescing immersion oil should be used and can also be placed between the condenser and the slide. Ordinary immersion oil should be avoided.

8. When electrons return to the ground state, the energy is not lost but can appear as a new form of energy such as heat, light, or chemical energy.

B. Multiple Choice Questions

1. a

2. a

3. e

4. a

5. c

**Exercise 5 (SV 4)**

**Microscopic Measurements**

A. Short Answer Questions

1. An ocular micrometer has lines separated by an arbitrary distance. A stage micrometer has lines separated by 0.01 mm and is used to calibrate the ocular micrometer.

2. If 2 stage divisions = 0.02 mm = 13 ocular divisions, then each ocular division equals 0.0015 mm or 1.5 microns (0.02/13). A cell that spans 16 ocular divisions would be (16\*0.0015) 0.025 mm or 25 microns in diameter.

3. Calibration is necessary for each objective because of the differences in magnification (distances between lines of stage micrometer changes).

**Exercise 6 (SV 5)**

**Microbiology of Pond Water—Protists, Algae, and Cyanobacteria**

A. Results

1. Sketches of protozoa

2. Sketches of algae

3. Sketches of cyanobacteria

B. Short Answer Questions

1. Algae and protozoa are classified in the domain Eukarya. Cyanobacteria are classified in the domain Prokarya.

2. a. Algae and plants are both photosynthetic and have cell walls composed of cellulose.

b. Algae, however, can be single-celled and motile.

3. Protozoa utilize flagella, cilia, or pseudopodia (amoeboid movement) for motility. Flagella and cilia are fibrous, extracellular, protein appendages that wave. Flagella are longer and singular whereas cilia are shorter and more numerous. Pseudopodia are cytoplasmic projections that enable a “crawling” motion.

4. a. Cyanobacteria

b. Cyanobacteria are prokaryotic cells that, unlike eukaryotic algae, lack a nucleus and chloroplasts, although they do contain chlorophyll.

5. Overgrowths or blooms of microscopic algae cause “Red Tides” and the cellular pigments are responsible for the oceans taking on a red color.

6. Malaria is caused by the genus *Plasmodium,* a Protista found in the group Apicomplexa.

7. Mitosomes are reduced mitochondria that lack electron transport components. They occur in the dipomonads of which the human pathogen, *Giardia lambdia,* is a member.

8. A kinetoplast is a large mass of DNA in the mitochondrion of a kinetoplastid such as the trypanosomes, which cause African sleeping sickness and Chagas disease.

9. Sacs called alveoli in the cytoplasmic membrane are thought to control osmotic balance.

10. Micronuclei contain DNA that encodes for genes concerned with sexual reproduction.

11. The frustule is the cell wall of diatoms. It consists of two halves that fit together like a box with a lid. Frustules are composed of silica.

12. In addition to chlorophyll, cyanobacteria have phycobiliproteins which are complexes of pigments and proteins. They also harvest light.

C. Fill-in-the-Blanks Questions

1. Protozoa = nucleus, flagella, cilia, pseudopodia

Algae = nucleus, flagella, photosynthetic pigment(s), chloroplasts, cell wall

Cyanobacteria = photosynthetic pigment(s), cell wall

2. Amoeboid cells = pseudopodia

Flagellates = flagella

Ciliates = Cilia

(the Diatom column was deleted from the text)

Apicomplexa = parasitic

3. Chloroplastida = chlorophyll a & b, flagella, cell wall, chloroplasts, starch

Chrysophyceae = chlorophyll a & c, fucoxanthin, cell wall, chloroplasts, oils, leucosin

Euglenozoa = chlorophyll a & b, flagella, chloroplasts, paramylon

Phaeophyceae = chlorophyll a & c, fucoxanthin, cell wall, chloroplasts, laminarin, mannitol

Bacillariophyta = chlorophyll a & c, fucoxanthin, cell wall, chloroplasts, laminarian, oils

Dinoflagellates = chlorophyll a & c, fucoxanthin, cell wall, chloroplasts, starch, oil, fat

Cyanobacteria = chlorophyll a, c-phycocyanin, c-phycoerythrin, cell wall

**Exercise 7 (SV 6)**

**Ubiquity of Bacteria**

A. Results

1. Colony counts

2. Variable answer

3. Variable; possible answers include, temperature, moisture, amount of human traffic.

4. (a) Variable answers (b) no/yes (c) Organisms sampled were not able to grow on the kind of nutrient agar used, or organisms sampled require longer to grow. (d) Students may feel an area sampled is sterile because it was recently cleaned.

B. Short Answer Questions

1. Bacterial colonies are generally smooth and small as compared to fungal colonies, which are large and “fuzzy.”

2. Since each colony is produced from a single cell, the number of colonies indicates the number of cells originally present or level of contamination. Colony size reflects growth rate.

3. Bacteria, such as the staphylococci and the diphtheroids, are part of the normal skin flora. Molds, however, are likely transient contaminants picked up from the environment.

4. Microbial levels on skin are best controlled by hand washing, on surfaces in the environment with use of disinfectants like bleach, and in the air by HEPA filtration systems.

5. a. Bacteria are smaller, about 0.5 to 10 µm in diameter.

1. Bacterial DNA is not enclosed in a nucleus but rather is organized in the cytoplasm.
2. Bacteria have 70S ribosomes.
3. Bacteria have a cell wall composed of peptidoglycan.
4. Bacteria lack mitochondria and chloroplasts but can carry out respiration and photosynthesis.
5. Bacteria may have flagella that are simpler in structure but may be more numerous.

**Exercise 8 (SV 7)**

**The Fungi: Yeasts and Molds**

A. Results

1. Yeast drawings and descriptions

2. Mold drawings and descriptions

B. Short Answer Questions

1. Coenocytic is a type of fungal mycelium that is not separated into individual cells by cross walls. Cellular organelles, nuclei, and cytoplasmic constituents move freely by cytoplasmic streaming.

2. Fungi have been traditionally classified using morphology and reproductive mechanisms. Modern approaches use genetic analysis which has shown that traditional approaches were not always correct in establishing the taxonomic groupings. However, identification still relies considerably on morphology.

3. Fungi contain chitin in their cell walls whereas plants contain cellulose.

4. The genus *Trichophyton* causes many infections in humans associated with hair, skin, and nails.

5. Dimorphic refers to fungi that grow as yeast forms in tissue and reproduce by budding, but produce mycelium and sporulation structures when grown in nutrient medium. *Histoplasma capsulatum* is an example of a dimorphic fungus.

6. Conidiospores are asexual spores that form on specialized hyphae called conidiophores. Zygospores are sexual spores that form by the fusion and genetic exchange between genetically distinct hyphae.

7. Fermentation by yeasts produce fermented beverages such as beer and wine. Yeast are also used in bread making, which is a fermentation process. Fungi are important in producing cheeses such as blue cheese and Roquefort. Mushrooms are used in a variety of foods such as pasta and pizza.

8. Mushrooms are usually edible whereas toadstools are poisonous.

9. The fungus *Claviceps* can infect grain, producing ergot alkaloids that are hallucinogenic. Some of the people involved in the Salem witch trial displayed symptoms characteristic of ergot poisoning that could have come from bread made with infected grain.

10. See answer 7.

11. The etomycorrhizae is formed by a close association between certain fungi and the roots of plants. The fungi facilitate the uptake of minerals and water by the plant and the plant supplies carbohydrates to the fungus for growth.

12. Wood-rotting fungi can degrade cellulose and lignin, a polymer of phenolic compounds.

**Exercise 9 (SV 8)**

**Aseptic Technique**

A. Results

1. Variable answer.

2. Success is presence of growth.

3. Failure is no growth; or growth of a wide variety of colonies, signaling contamination.

B. Short Answer Questions

1. When handling microbial cultures, aseptic technique limits contamination of yourself and your workspace with the microbes in the cultures, and it limits contamination of your cultures with unwanted environmental microbes.

2. The flame from a Bunsen burner is used to sterilize transfer instruments (e.g., inoculating loop) and is used to flame the opening of the tube after the cap is removed and before the cap is replaced.

3. Since the opening of a plate is not readily flamed, one should hold the lid over the top of the open plate when inoculating so that air contamination is limited. Working near a flame is also useful.

4. Labels should be written on the bottom of the agar plate.

5. Agar plates should be incubated in an inverted position to prevent condensation on the agar surface that could spread the inoculated organisms.

6. Disinfectants, such as bleach and alcohol, are generally useful against vegetative cells and viruses but may not completely eradicate bacterial endospores.

C. Multiple Choice Questions

1. e

2. b

3. c

**Exercise 10 (SV 9)**

**Pure Culture Techniques**

A. Results

1. Streak plate evaluation

2. Pour plate evaluation

3. Subculture evaluation

4. Variable, depends upon technique of the student. Beginning students generally have better separation with a pour plate.

5. Staining and microscopy or a streak plate can determine purity of a slant culture.

B. Short Answer Questions

1. A colony is a visible microbial growth on a solid medium that originated from a single parent and through cell division produced a multitude of identical daughter cells.

2. Useful colony characteristics for differentiation of bacterial species include size, color, shape, texture, opacity, and odor. For example, *Serratia marcescens* colonies are red, irregular in shape, and have a strong odor. *Micrococcus luteus* colonies, on the other hand, are yellow, opaque, round, and dome-shaped.

3. When working with culture that may contain millions of cells, dilution on to a solid medium is essential for separating the cell with enough space so that they grow into isolated colonies.

4. The streak plate method does not require any additional media for dilution and only requires one plate for inoculation.

5. The pour plate method requires less skill, has optimization built in, and will more likely produce the desired result.

6. The loop is flamed before entering a culture tube to ensure that no contaminating microbes are introduced into the culture. The loop is flamed afterward so that no culture microorganisms are introduced into the working environment.

7. Molten agar must be cooled to 50⁰C so that microbes added to the medium will not be killed by excessive heat, but it must not be cooled too much because it will solidify before the cells can be dispersed and poured. Also, if too hot when poured, excess moisture will form on the lid of the plate.

8. Plates are inverted during incubation so that moisture does not accumulate on the lid and drop on to the agar surface. This will cause the organisms to spread and negate the dilution effect. Also, agar plates tend to dehydrate faster in the upright position.

**Exercise 11 (SV 10)**

**Smear Preparation**

1. To prepare cells from solid media, cells must be mixed in 1–2 drops of water on the slide. Two to three loopfuls of cells from liquid media can be transferred directly to the slide.

2. Large amounts of cells in a smear can cause staining artifacts because stain is not washed away by destaining agents or water.

3. Heat fixation causes cells to adhere to the slide during staining. However, structures such a capsules can undergo shrinkage during heat fixations and be lost in staining procedures. For that reason capsule stains are not normally fixed.

**Exercise 12 (SV 11)**

**Simple Staining**

A. Results

1. Pleomorphism, metachromatic granules, palisades arrangement (drawing)

2. Streptococci are ovoid cells that occur in pairs and chains.

3. Staphylococci are cocci that occur in grapelike clusters.

4. Bacillus are rods that can occur singly or in chains.

B. Short Answer Questions

1. Chromophores are color-bearing groups of stains.

2. Acidic dyes are negatively charged and therefore repelled by negatively charged bacterial cells.

3. Crystal violet is a basic stain which has a positive charge.

4. Volutin is polyphosphate.

5. A palisade arrangement is a parallel arrangement of rod-shaped cells characteristic of the *Corynebacteria.* Darkfield microscopy of unstained cells creates and image most similar to negative staining.

6. Basic dyes are positively charged and therefore attracted to negatively charged bacterial cells. Acidic dyes are negatively charged and therefore repelled by bacterial cells.

**Exercise 13 (SV 12)**

**Negative Staining**

A. Results

1. Streptococci are ovid cells that occur in pairs or chains; yeasts are large ovid cells that have characteristic buds and are much larger than bacteria; spirochaetes are spiral-shaped cells that are very thin and usually not seen unless darkfield microscopy is used.

B. Short-Answer Questions

1. Negative stains have negatively charged chromophores and are repelled by negatively charged bacterial cells.

2. Nigrosin and India ink are examples of negative stains.

3. Heat fixation is normally omitted when determining dimensions of bacterial cells because heat will cause cells to shrink.

4. Negative stains can demonstrate capsules.

**Exercise 14 (SV 13)**

**Capsular Staining**

A. Results

1. Capsule stains do not penetrate the capsule but rather form an opaque background surrounding the cell, highlighting the presence of the capsule.

B. Short-Answer Questions

1. The capsule can inhibit phagocytosis of pathogens, such as *Streptococcus pneumoniae* by white blood cells. The capsule also facilitates the attachment of bacterial cells to solid surfaces.

2. Capsules are composed of polysaccharides or proteins.

3. *Streptococcus mutans* forms a capsule that allows the bacterium to attach to the surface of a tooth. This results in the formation of a biofilm called plaque.

4. Heat causes the capsule to shrink because of the loss of water. Therefore the student will probably not see a capsule in their stain.