HT Practice Test

1. Heat fixation should NOT be used for which of the following stains?

a. Capsular staining
b. Gram stain
c. Endospore staining
d. Acid-fast stain

2. Which of the following is an example of a noncoagulant fixative?

a. Picric acid
b. Zinc salts
c. Ethanol
d. Formaldehyde

3. Which of the following methods could be used to remove mercury pigment, a fixation artifact?

a. Treat the specimen with an iodine solution followed by bleaching with sodium thiosulfate
b. Treat the specimen with saturated alcoholic picric acid
c. Treat specimen with 10% ammonium hydroxide in 70% ethyl alcohol
d. Treat the specimen with 1% acid alcohol

4. Which of the following fixative reagents causes tissue swelling?

a. Mercuric chloride
b. Picric acid
c. Acetic acid
d. Ethanol

5. Which of the following fixative reagents does NOT cause tissue hardening?

a. Picric acid
b. Formalin
c. Acetone
d. Mercuric chloride

6. Which of the following is NOT a primary purpose of fixation?

a. To prevent putrefaction
b. To prevent autolysis
c. To enhance differences in the refractive indexes of various tissue structures
d. To expose antigen sites for immunohistochemical staining

7. Autolysis is defined as the following:

a. Postmortem decay caused by bacteria
b. Denature of proteins in the tissue caused by chemical fixation
c. The process of removing calcium from bone or tissue
d. Destruction of tissues by enzymes normally present in the cells

8. When processing delicate specimens using a standard closed tissue processor, dehydration should be done by which of the following methods to minimize tissue distortion?

a. A graded series of reagents of increasing concentration
b. A graded series of reagents of decreasing concentration
c. A single reagent at a single concentration
d. Delicate specimens do not require a dehydration step

9. When preparing a sample for electron microscopy, which of the following embedding materials should be used?

a. Paraffin
b. Agar
c. Gelatin
d. Resin

10. Which of the following is NOT an advantage of tissue processing using a microwave oven?

a. Shorter processing time
b. Does not require monitoring, calibration, or manual transfer of tissues
c. Does not require graded concentrations of solutions
d. Does not require the use of xylene, which eliminates the associated toxic fumes

11. Which of the following is a "universal solvent"?

a. Isopropyl alcohol
b. Dioxane
c. Toluene
d. Acetone

12. Which of the following is an artifact of over-decalcification of bone tissue?

a. The slide appears to be covered in dust
b. Hematoxylin and eosin (H&E) stain shows poor nuclear (basophilic) staining
c. Large holes are present that could be mistaken for vacuoles
d. The tissue shows a "parched earth" cracking appearance

13. When orienting a tissue for embedding, which of the following tissues requires special attention to ensure it is cut in cross section?

a. Brain
b. Liver
c. Fallopian tubes
d. Muscle biopsies

14. Which of the following should be used for sectioning celloidin?

a. Rotary microtome
b. Sliding microtome
c. Clinical freezing microtome
d. Retracting microtome

Answer Key and Explanations

1. A: Most bacteria produce a capsule, or glycocalyx, just outside the cell wall. This capsule is usually made up of polysaccharides. Heat fixation will cause this moist slime layer to shrink, making it difficult to see once stained. Also, heat fixing may cause a bacterial cell to shrink, creating a clear zone around the cell that appears like a capsule when one does not truly exist. Therefore, when staining to view a bacterial capsule, a sample is air-dried and then a negative stain is generally used for visualization.

2. D: Coagulant fixatives allow solutions to readily enter into the interior of the tissue, but they destroy or distort cytoplasmic organelles such as mitochondria and lysosomes. Noncoagulant fixatives, such as formaldehyde, cross-link the structural macromolecules of the tissue, creating a gel that preserves organelles well but inhibits the penetration of solutions into the tissue.

3. A: Mercury pigment can be removed by treating the specimen with an iodine solution followed by bleaching with sodium thiosulfate. Formalin pigment and malarial pigment can both be removed by either treating the specimen with saturated alcoholic picric acid or by treating the specimen with 10% ammonium hydroxide in 70% ethyl alcohol. Chromic oxide pigment can be removed using 1% acid alcohol.

4. C: Acetic acid causes swelling of tissue. On the other hand, acetic acid, mercuric chloride, and ethanol all cause tissues to shrink. Bouin solution, a fixative compound, balances these effects by combining acetic acid with picric acid.

5. A: Formalin, acetone, and mercuric chloride all cause tissue hardening; therefore, it is important to make sure the fixation time is not prolonged when using these reagents to prevent tissues from becoming too brittle.

6. D: Fixation has many purposes including preventing autolysis and putrefaction, enhancing differences in refractive indexes of tissue structures, maintaining proper relationship between cells and extracellular substances, and making the tissue firmer so dissection and cutting is easier. However, fixation can have the downside of masking antigenic sites, resulting in poor immunohistochemical staining.

7. D: Autolysis, or the destruction of tissues by enzymes, can continue to occur even after the blood supply to the tissue has been cut off. Fixatives prevent autolysis. Autolysis is more severe in tissues that contain high enzyme levels such as the liver, brain, and kidneys. Areas of the tissue that have undergone autolysis will stain poorly.

8. A: Dehydration should be done slowly. If the concentration gradient differs significantly between the inside and the outside of the tissue, the resulting diffusion currents could produce cell distortions. This is why slowly replacing the water through a graded series of reagents of increasing concentration is necessary to maintain proper structure before clearing and subsequent infiltration with a medium such as paraffin.

9. D: Resins are the only embedding material used for electron microscopy. Resins are harder than wax; therefore, it is possible to cut the ultrathin sections commonly used for electron microscopy.

10. B: Microwave ovens increase the internal heat of specimens, thus accelerating reaction times, so solutions diffuse into tissues more quickly. Also, only one dehydrating step is necessary.

Combined, these factors decrease the overall time of processing. The use of more environmentally friendly reagents is another benefit of using microwave ovens for processing. One disadvantage, though, is the need to manually transfer the tissue from one reagent to the next.

11. B: A universal solvent is a chemical that can be used for both the dehydrating and clearing steps. Examples of universal solvents include dioxane, tertiary butanol, and tetrahydrofuran.

12. B: The most common problems associated with bone processing are bone dust, under-decalcification, and over-decalcification. When dust created by the saw when sectioning is pressed into the surface of the bone, the resulting slide appears to be covered in dust. Using a saw with a diamond blade can prevent this problem. Under-decalcification makes section cutting very difficult, resulting in fragmentation problems. Over-decalcification results in poor nuclear staining.

13. C: While most tissues are embedded flat, some tissues require special orientation. Tubular structures, such as fallopian tubes, should be embedded in cross section so that the lumen and all layers can be seen. Tissues with an epithelial surface, such as skin, are oriented so that they are cut in a plane at a right angle to the surface.

14. B: Routine paraffin sections and frozen sections are generally cut using a rotary microtome, which is also the type found in most cryostats. Sliding microtomes are used for cutting celloidin and large paraffin blocks. The clinical freezing microtome, now replaced in most cases by the cryostat, is used for preparing frozen sections. Finally, the retracting microtome is used for cutting plastic sections.

15. A: Agar, gelatin, or Elmer's glue can be added to a water bath to increase adhesion. Triton X-100, Brij-35, and 95% alcohol can be added to a water bath to decrease the wrinkles in a paraffin section.

16. D: Most unfixed tissues should section well at -15° to -23°C. Adipose tissue (or fat tissue) does not freeze well at this temperature range. Therefore, the temperature must be lowered to a range of -25° to -30°C in order to make the fat tissue hard enough to section well.

17. B: Steel knives have been replaced with disposable blades for routine microtomy, although a few exceptions may still exist. Glass and diamond knives are used for electron microscopy to cut tissues embedded in plastic.

18. B: Many problems can occur during microtomy. When sectioned too aggressively, tissues can have a moth-eaten appearance, meaning they have many holes throughout. Brain, liver, and lymph nodes are especially prone to this artifact. Other factors that can adversely affect the outcome of tissue sectioning include improper clearance angle of the knife and blade dullness or nicking.

19. C: Most paraffin-embedded sections are cut to be 3 to 5 µm thick. Resin sections for electron microscopy are cut at a thickness of 0.5 to 1 µm for tissue orientation prior to thin sectioning of tissues at 50 to 90 nm.

20. C: Birefringent materials such as amyloid can split a ray of light into two separate waves that are refracted in different directions. A polarizing microscope has two polarizing filters, the polarizer and the analyzer. When one filter is oriented east-west and the other north-south, then no light will appear, but birefringent materials can be seen when rotated between these two filters.