LESSON 8-2  Collecting and Processing Specimens for Parasite Examination
Student Performance Guide

LESSON 8-3  Microscopic Methods for
Student Performance Guide

LESSON 8-4 Preparing and Staining Smears for Blood Parasites
Student Performance Guide
INSTRUCTIONS
1. Practice the procedures for collecting and processing specimens for parasite examination, following the step-by-step procedure.
2. Demonstrate your understanding of this lesson by:
   a. Completing a written examination successfully, and
   b. Performing the procedures for collecting and processing specimens for parasite examination satisfactorily for the instructor. All steps must be completed as listed on the instructor’s Performance Check Sheet.

MATERIALS AND EQUIPMENT
- gloves
- hand disinfectant
- 10% chlorine bleach solution
- wooden tongue depressors
- wooden applicator sticks
- microscope slides
- clear cellophane tape
- xylene or toluene
- microscope
- biohazard disposal container
- sharps disposal container
- leakproof vials for transporting specimens
- 10% formalin
- PVA (or other appropriate) fixative
- fecal specimens (students may bring pet specimens for practice)
- fecal specimen containers
- atlas of parasitic morphology containing illustrations

PROCEDURE
Record in the comment section any problems encountered while practicing the procedure (or have a fellow student or the instructor evaluate your performance).

You must:

1. Instruct a patient in the proper procedure for collecting a fecal specimen (steps 1a–c):
   a. Give patient a fecal specimen container with lid and label
   b. Explain the fecal collection procedure to the patient, emphasizing the following precautions:
      (1) Specimen must not be contaminated with urine or water
      (2) Outer surface of specimen container must not be contaminated
      (3) Container must be labeled with the patient’s name, date, and time of specimen collection

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<th>You must:</th>
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S = Satisfactory
U = Unsatisfactory
### You must:

1. Prepare a fecal specimen for transport (steps 2a–i)
   - Wash your hands and put on gloves
   - Obtain a fecal specimen, fixative vials, and transport containers.
     - Check label to be sure required patient information is present (name, date, time of collection)
   - Open container and observe consistency of specimen. Record as watery, loose, soft, or formed
   - Use disposable applicators to obtain a portion of specimen approximately one-third the volume of the fixative
   - Place specimen in vial and mix thoroughly using applicator. Discard applicator(s) into biohazard container. Label vial with patient information
   - Repeat procedure (steps 2e–f) with the second vial of fixative, using clean applicators
   - Disinfect work area with surface disinfectant
   - Remove and discard gloves in biohazard container and wash your hands with hand disinfectant

2. Prepare a cellophane tape perianal swab (steps 3a–e)
   - Obtain tape, clean microscope slide, and paper tab
   - Attach a four-to-five-inch section of tape to one end of the microscope slide’s back
   - Fold the tape around the end of the slide and smooth down over the slide top, so the tape adheres to the slide, leaving a small portion free at the end
   - Attach a small paper tab to the free end of the tape to use as a label and lifting tab
   - Store the prepared slide in a cool, dust-free location

3. Demonstrate the use of the cellophane tape swab (steps 4a–k)
   - Wash your hands and put on gloves
   - Obtain a clean wooden tongue depressor
   - Obtain previously prepared cellophane tape slide (step 3) and label the tab
   - Place the cellophane tape swab near one end of the depressor, tape side up
   - Lift the cellophane tape and form a loop around the end of the tongue depressor, sticky side of tape to the outside
   - Explain how to obtain the pinworm specimen by touching sticky surface of the tape to perianal region

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Estridge, B., Reynolds, A., and Walters, N. *Basic Medical Laboratory Techniques*. © 2000 Delmar, a division of Thomson Learning
### You must:

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<tr>
<td>g. Remove the tape from the tongue depressor and smooth back into place on the microscope slide, being careful not to touch the sticky surface</td>
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<tr>
<td>h. Discard the tongue depressor in biohazard container (if actually used for specimen collection)</td>
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<tr>
<td>i. Place the slide on a microscope stage and demonstrate how to observe for eggs using the low-power (10X) objective</td>
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<td>j. Clear glue from tape by placing a drop of xylene or toluene under the tape if desired (follow proper precautions in handling these chemicals)</td>
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<td>k. Remove the slide from the microscope stage and discard in biohazard sharps container</td>
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5. Discard specimen in appropriate biohazard container, as directed by instructor

6. Discard used materials appropriately

7. Return supplies to storage

8. Disinfect work area with surface disinfectant

9. Remove and discard gloves in biohazard container and wash your hands with hand disinfectant

**Evaluator Comments:**

Evaluator ___________________________ Date ________________
INSTRUCTIONS

1. Practice preparing fecal specimens for microscopic examination for intestinal parasites, following the step-by-step procedure.

2. Demonstrate your understanding of this lesson by:
   a. Completing a written examination successfully,
   b. Preparing fecal specimens for microscopic examination for intestinal parasites satisfactorily for the instructor. All steps must be completed as listed on the instructor’s Performance Check Sheet.

MATERIALS AND EQUIPMENT

- gloves
- hand disinfectant
- surface disinfectant (10% chlorine bleach solution)
- absorbent lab paper
- puncture-proof container for sharps
- biohazard containers
- wooden applicator sticks
- fecal specimens preserved in 10% formalin and in PVA (specimens from pets may be used)
- fume hood

Materials for Wet Mounts:
- coverglasses, 22 mm²
- glass microscope slides, 2 x 3 inch preferable
- saline (0.85% NaCl)

- iodine solution for fecal wet mounts (Lugol’s, Dobell and O’Connors, or D’Antoni’s)
- dropping pipets
- D’Antoni’s Iodine:
  Add 1.0 g potassium iodide (KI) and 1.5 g iodine crystals to 100 mL distilled water in a dark bottle. Shake well and filter daily before use.

Materials for Preparing Fecal Smear for Staining:
- 35°C incubator or laboratory oven
- microscope slides, 1 x 3 inch
- wooden applicator sticks

Materials for Formalin-Ethyl Acetate Sedimentation (or a commercial stool concentration kit):
- conical centrifuge tubes, 15 mL capacity (preferably disposable)
- 10% formalin
- ethyl acetate
- cotton swabs
- gauze or cheesecloth
- plastic funnel
- clinical centrifuge capable of spinning 15 mL conical tubes at 500 xg
- wooden applicator sticks

Optional: Preserved fecal specimens containing parasites, atlases, diagrams, stained microscope slides, photographs, or videos of intestinal parasites
### PROCEDURE

Record in the comment section any problems encountered while practicing the procedure (or have a fellow student or the instructor evaluate your performance).  

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<td>1. Assemble equipment and materials</td>
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<td>2. Wash your hands and put on gloves</td>
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<td>3. Prepare a work area by placing absorbent lab paper on counter or in fume hood</td>
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<td>4. Demonstrate the procedure for preparing a fecal smear for staining, following steps 4a–d</td>
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<td>a. Obtain PVA-fixed fecal specimen and two 1 x 3 inch microscope slides</td>
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<td>b. Use applicator stick to mix specimen and remove a portion</td>
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<td>c. Spread specimen evenly on slide by rolling stick across slide or smearing in a zig-zag fashion (see Figure 8-11). Smear should cover one-third to one-half the length of the slide and should extend from the slide’s top to bottom edges. Prepare a second slide from the same specimen</td>
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<td>d. Label slides and place them in 35°C incubator to dry for at least four hours, preferably overnight</td>
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<td>5. Demonstrate the procedure for preparing saline and iodine wet mounts from a formalin-fixed fecal specimen, following steps 5a–i</td>
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<tr>
<td>a. Obtain a formalin-fixed specimen</td>
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<td>b. Place a 2 x 3 inch glass slide on work area</td>
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<tr>
<td>c. Place one drop of saline on left half of slide and one drop of iodine solution on right half of slide</td>
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<tr>
<td>d. Use applicator stick to mix specimen</td>
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<tr>
<td>e. Remove a small portion of specimen with applicator stick and mix in with saline drop. Place a coverglass over the drop</td>
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<td>f. Remove another small portion of fecal specimen and mix in with iodine drop. Place a coverglass over the drop. Discard applicator in biohazard container</td>
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<td>g. Place slide over newsprint and check thickness of wet mounts (letters should be readable through the specimen)</td>
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<td>h. <strong>Optional:</strong> Place slide on microscope stage and scan specimens with the low-power and high-power objectives. Use visual aids such as atlases, diagrams, or photographs to help recognize parasitic forms</td>
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<tr>
<td>i. Discard slide in biohazard sharps container</td>
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### You must:

6. Demonstrate the procedure for formalin-ethyl acetate sedimentation, following steps 6a-k, or skip to step 7
   a. Obtain a formalin-fixed fecal specimen
   b. Mix the specimen and strain 5mL through wet gauze into a conical centrifuge tube
   c. Add saline or formalin to a volume of 15 mL and centrifuge the tube at 500 xg for 10 min
   d. Decant supernatant into a container of disinfectant, leaving approximately 1mL in tube
   e. Add 9 mL of 10% formalin to tube and mix using applicator stick
   f. Add 4 mL ethyl acetate, cap tube securely, and shake it vigorously by inversion for one-half minute
   g. Centrifuge tube for ten minutes at 500 xg
   h. Use a wooden applicator to ring the debris plug
   i. Pour off the top layers of ethyl acetate, debris, and formalin (see Figure 8-10), leaving 0.5–1.0 mL of sediment in tube
   j. Use a cotton swab to remove debris from the inside of tube. Add a few drops of 10% formalin to resuspend sediment
   k. Mix sediment with applicator stick and prepare saline and iodine wet mounts from portions of sediment, as in step 5b–i

7. Discard all contaminated materials in appropriate biohazard containers

8. Discard or store preserved specimens, as directed by instructor.

9. Disinfect work area and reusable supplies with 10% chlorine bleach solution

10. Remove and discard gloves in biohazard container and wash your hands with hand disinfectant

**Evaluator Comments:**

Evaluator __________________________________________ Date _________________________
LESSON 8-4 Preparing and Staining Smears for Blood Parasites

Name _________________________________________________________________ Date ______________________

INSTRUCTIONS

1. Practice preparing and staining smears for blood parasites, following the step-by-step procedure.
2. Demonstrate your understanding of this lesson by:
   a. Completing a written examination successfully, and
   b. Performing the procedure for preparing and staining smears for blood parasites satisfactorily for the instructor. All steps must be completed as listed on the instructor’s Performance Check Sheet.

MATERIALS AND EQUIPMENT

- gloves
- hand disinfectant
- surface disinfectant
- biohazard container
- puncture-proof container for sharp objects
- acrylic safety shield or face protection
- Materials for capillary puncture
- clean glass slides
- absolute methanol
- stock Giemsa stain
- staining jars (Coplin jars)
- phosphate-buffered water, pH 7.2
  Recipe for Giemsa buffer (phosphate-buffered water):
  39 mL 0.067 M NaH₂PO₄
  61 mL 0.067 M Na₂HPO₄
  900 mL distilled water
  check pH. Should be 7.0–7.2
- microscope
- immersion oil
- lens paper
- slide box

Optional visual aids: commercially prepared stained slides of Plasmodium and Babesia; commercially prepared stained slides of filarial worms, such as dog heartworm (Dirofilaria sp.); charts, Kodachrome slides, and figures showing morphology of blood parasites

Note: Consult manufacturer’s instructions accompanying Giemsa stain for recommended optimal dilution and staining time.

PROCEDURE

Record in the comment section any problems encountered while practicing the procedure (or have a fellow student or the instructor evaluate the performance).

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<td>2. Assemble equipment and materials for capillary puncture</td>
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<td>3. Perform a capillary puncture (as described in Lesson 2-2)</td>
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<td>4. Wipe away the first drop of blood</td>
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<td>5.</td>
<td>Apply a small drop of blood to a clean glass slide and use a clean spreader slide to form a thin blood film (as in Lesson 2-8). Make duplicate smears and set slides aside to air-dry.</td>
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<td>6.</td>
<td>Immerse dried thin smears in absolute methanol for thirty to sixty seconds and air-dry.</td>
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<td>7.</td>
<td>Make a thick smear by holding a clean slide under the patient's finger and allowing one or two large drops of blood to fall on the center of the slide.</td>
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<td>8.</td>
<td>Spread the blood evenly into a dime-sized circle to form the thick smear, using slight pressure against the fingertip (or use the corner of a clean glass slide to spread the blood). Make duplicate smears.</td>
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<td>9.</td>
<td>Check the thickness of the thick smears by laying the slides on printed material. The print should be readable through the blood film.</td>
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<td>10.</td>
<td>Place the slides on a flat surface in a dust-free place and allow them to air-dry at room temperature for several hours or overnight. DO NOT FIX.</td>
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<td>11.</td>
<td>Have patient apply pressure to puncture site with sterile gauze when satisfactory smears have been obtained. (At this point, if staining is to be done another day, work area may be cleaned, gloves removed, and hands washed, steps 20-25. Reglove before handling smears for staining procedure)</td>
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<td>12.</td>
<td>Stain smears by immersing slides in a freshly prepared 1:50 dilution of Giemsa stain for fifty minutes. (Be sure thin smear has been fixed and thick smear has dried for several hours)</td>
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<td>13.</td>
<td>Rinse stained smears in buffered water: rinse thin smear one to two minutes; rinse thick smear three to five minutes.</td>
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<td>14.</td>
<td>Place rinsed slides in slide rack and allow to air-dry.</td>
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<td>15.</td>
<td>Place thin smear on microscope stage and observe cells using oil-immersion objective. Observe quality of stain: RBCs should be pinkish, WBC nuclei should be blue-purple. Examine RBCs for stained intracellular parasitic inclusions. (Refer to charts, figures, or commercially prepared slides, if available)</td>
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<td>16.</td>
<td>Remove thin smear from microscope stage and place thick smear in position.</td>
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<td>17.</td>
<td>Examine thick smear using oil-immersion objective. WBCs and platelets should be visible, but RBCs should have been destroyed in the staining process. If parasites were present in the blood specimen, they will be stained. (Refer to charts, figures, or commercially prepared slides, if available)</td>
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<td>18.</td>
<td>Remove slide from microscope stage.</td>
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<td>19.</td>
<td>Clean oil from microscope objective with lens paper.</td>
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<td>20. Place slides in covered slide box</td>
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<td>21. Discard capillary puncture materials in appropriate biohazard</td>
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<td>containers</td>
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<td>22. Clean equipment and return to proper storage</td>
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<td>23. Clean work area with surface disinfectant</td>
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<td>24. Remove and discard gloves in biohazard container</td>
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<td>25. Wash your hands with hand disinfectant</td>
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_Evaluator Comments:

Evaluator __________________________________________ Date ______________